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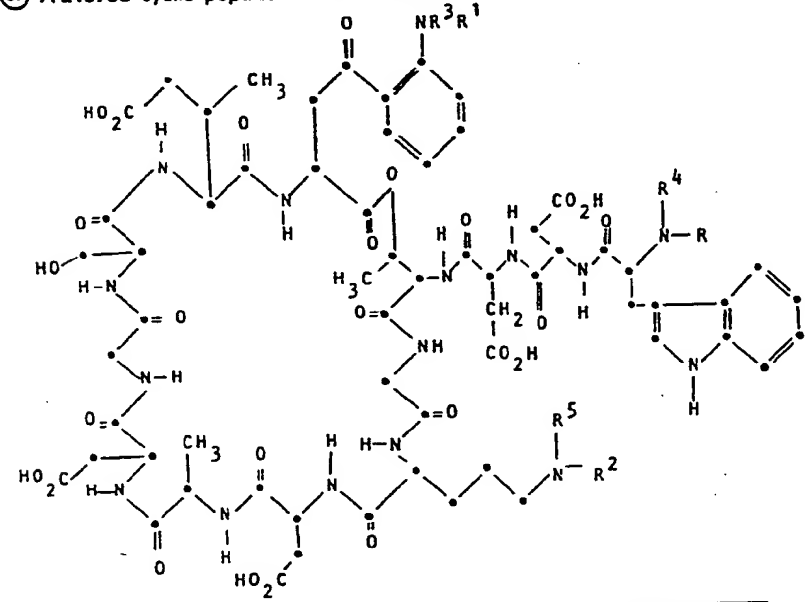
(54) **Cyclic peptide derivatives.**

(57) A-21978C cyclic peptides of the formula



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wherein R is selected from the group consisting of hydrogen, an amino-protecting group, 8-methyldecanoyl, 10-methylundecanoyl, 10-methyldodecanoyl, the specific C_{10}-alkanoyl group of A-21978C factor C_0 and the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5; R^1 and R^2 are, independently, hydrogen or an amino-protecting group, and salts thereof, are prepared by enzymatic deacylation of an antibiotic selected from A-21978C complex, A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5 and blocked A-21978C complex and factors C_0, C_1, C_2, C_3, C_4 and C_5, using an enzyme produced by the *Actinoplanaceae*, preferably by *Actinoplanes utahensis*. These A-21978C cyclic peptides or a pharmaceutically-acceptable salt thereof are useful intermediates for preparing new alkanoyl, alkenoyl, benzoyl, acylamino, N-alkanoylaminoacyl, and alkyl cyclic peptide antibiotics.

CYCLIC PEPTIDE DERIVATIVES

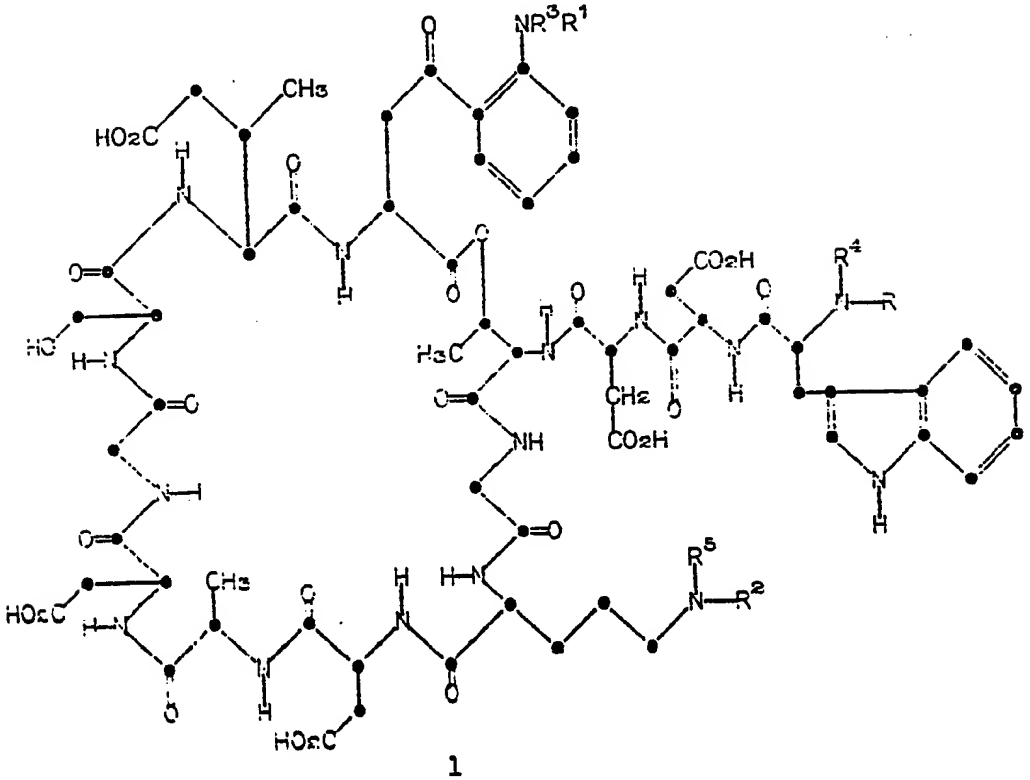
This invention relates to novel derivatives of cyclic peptides which possess antibiotic properties and to the method of producing these antibiotic derivatives by semisynthetic means.

There is a great need to develop new antibiotics because of the great possibility and constant threat that antibiotic-specific resistant strains of pathogenic microorganisms will develop. In particular, pathogens within the gram positive genera Staphylococcus and Streptococcus often are resistant to commonly used antibiotics such as penicillin and erythromycin; see for example, W.O. Foye, Principles of Medicinal Chemistry, pp. 684-686 (1974).

In accordance with the invention, new derivatives of the A-21978C cyclic peptides and the pharmaceutically-acceptable salts thereof, have been found to be effective antibiotics.

These new derivatives can be prepared by reacting the A-21978C nucleus or protected derivatives thereof, prepared by deacylating the appropriately blocked A-21978C complex or any of its appropriately blocked individual factors C_0, C_1, C_2, C_3, C_4, or C_5, with the desired acylating agent or an activated derivative thereof.

In accordance with the invention, A-21978C cyclic peptides of formula 1:



1

in which R, R^1, and R^2 are, independently, hydrogen, 8-methyldecanoyl, 10-methyldodecanoyl, 10-methylundecanoyl, the specific C_{10}-alkanoyl group of A-21978C_0 the specific C_{12}-alkanoyl groups of A-21978C factors C_4 or C_5, an amino-protecting group,

(A) an aminoacyl group of the formula

in which Q is C_1-C_{16} alkylene, or an N-alkanoyl-aminoacyl group of the formula

$$-W-\overset{\overset{O}{\|}}{C}-R^7$$

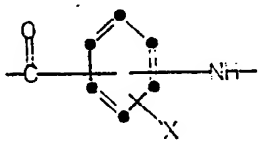
in which W is a divalent aminoacyl radical of the formula:

(1) $-\overset{\overset{O}{\|}}{C}-A-NH-$

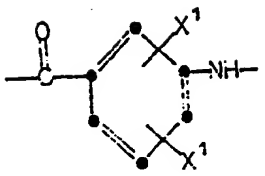
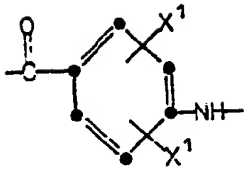
in which A is C_1-C_{10} alkylene or C_5-C_6 cycloalkylene;

(2) $-\overset{\overset{O}{\|}}{C}-\overset{\overset{R^8}{|}}{C}H-NH-$

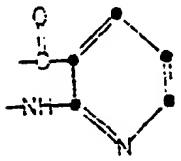
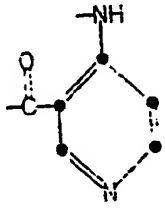
in which R^8 is hydroxymethyl, hydroxyethyl, mercaptomethyl, mercaptoethyl, methylthioethyl, 2-thienyl, 3-indole-methyl, phenyl, benzyl, or substituted phenyl or substituted benzyl in which the benzene ring thereof is substituted with chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3-alkoxy, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

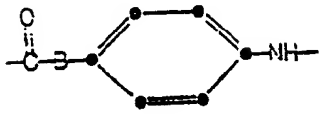
(3) 

in which X is hydrogen, chloro, bromo, iodo, amino, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy, mercapto, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

(4)  or 

in which X^1 is chloro, bromo, iodo, amino, hydroxy, C_1-C_3 alkyl or C_1-C_3 alkoxy;

(5)  or  ; or

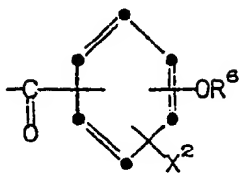
(6) 

in which B is a divalent radical of the formula: $-(CH_2)_n-$ and n is an integer from 1 to 3; $-CH=CH-$; $-CH=CH-CH_2-$; or

$-CH_2-NH-\overset{\overset{O}{\|}}{C}-$;

R^7 is C_1-C_{17} alkyl or C_2-C_{17} alkenyl;

(B) a substituted benzoyl group of the formula



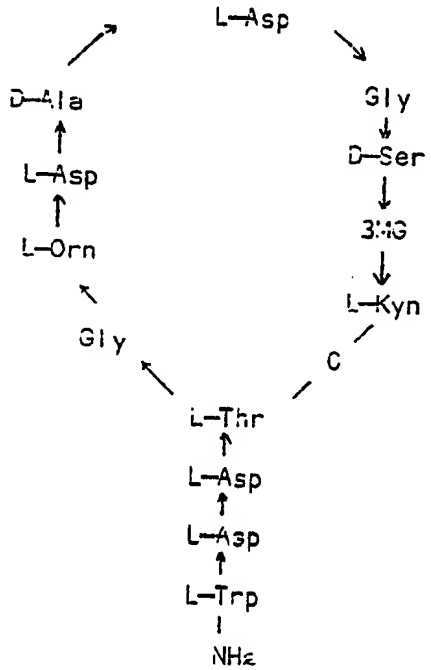
in which R^6 is C_8-C_{15} alkyl;
X^2 is hydrogen, chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy or C_1-C_3 alkylthio;

(C) optionally substituted C_2-C_{19} alkanoyl, C_5-C_{19} alkenoyl, C_4-C_{14} alkyl provided that R, R^1 and R^2 groups together must contain at least four carbon atoms;

and in which R^3, R^4 and R^5 (i) may represent hydrogen or (ii) taken together with an appropriate adjacent R, R^1, R^2 group may represent a C_4-C_{14} alkylidene group; provided that when R^1 and R^2 are both selected from hydrogen, R cannot be 8-methyldecanoyl, 10-methyl-undecanoyl, 10-methyldodecanoyl, the specific C_{10}-alkanoyl group of A-21978C factor C_0 or the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5; or a pharmaceutically-acceptable salt thereof, are useful in the preparation of, or as semi-synthetic antibiotics.

The compounds of formula 1 in which R is 8-methyldecanoyl, 10-methylundecanoyl, 10-methyldodecanoyl, the C_{10}-alkanoyl group of A-21978C factor C_0 and the C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5 and one of R^1 or R^2 is an amino-protecting group are blocked antibiotic A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5. These blocked antibiotic compounds are useful intermediates to certain peptides of formula 1, i.e. those in which R is hydrogen and at least one of R^1 and R^2 is an amino-protecting group.

The compound of formula 1 in which R, R^1 and R^2 each represent hydrogen is the common cyclic peptide present in antibiotic A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5. For convenience, this compound will be called the A-21978C nucleus. This compound can be represented also by formula 2:



2

in which 3MG represents L-threo-3-methylglutamic acid.

In another aspect, this invention relates to a method of enzymatically deacylating an antibiotic selected from A-21978C complex, A-21978C factors C_0, C_1, C_2, C_3, C_4, and C_5, blocked A-21978C complex, and blocked A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5. The naturally occurring A-21978C factors have a common cyclic peptide nucleus, but each has a different fatty acid group attached to the α-amino group of the tryptophan residue. The method of removing the fatty acid groups from the A-21978C factors and blocked factors provided by this invention comprises exposing the antibiotic or blocked antibiotic, in an aqueous medium, to an enzyme produced by a microorganism of the family Actinoplanaceae until substantial deacylation is accomplished.

A preferred method of this invention comprises using an enzyme produced by the microorganism Actinoplanes utahensis NRRL 12052 to cleave the fatty acid side chain. Deacylation is ordinarily accomplished by adding the appropriate antibiotic or blocked antibiotic to a culture of A. utahensis and permitting the culture to incubate until deacylation is accomplished. The A-21978C cyclic peptide or blocked peptide thereby obtained is separated from the fermentation broth by known methods.

The A-21978C nuclei are useful in that they can be reacylated to provide new antibiotic substances. These new substances are described in detail later.

The accompanying infrared absorption spectra, run in KBr, represent the following:

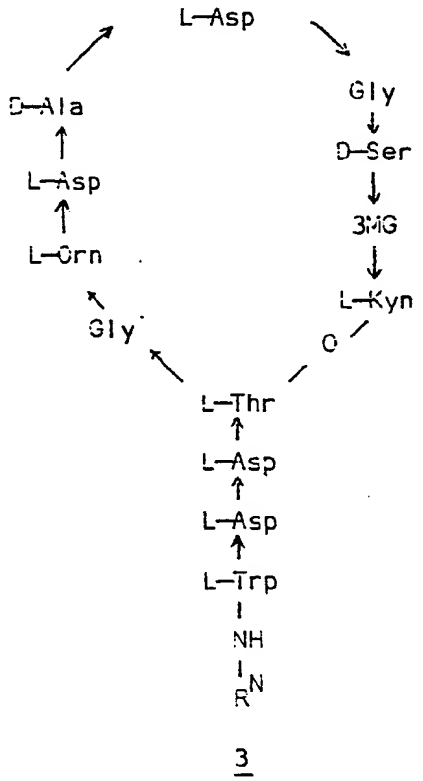
Figure 1 - A-21978C nucleus (formula 1, R, R^1, R^2 = H)

Figure 2 - A-21978C N_{Orn}-t-BOC nucleus (formula 1, R and R^1 = H; R^2 = tert-butyloxycarbonyl)

In this specification the following abbreviations, most of which are commonly known in the art, are used:

Ala:	alanine
Asp:	aspartic acid
Gly:	glycine
Kyn:	kynurenine
Orn:	ornithine
Ser:	serine
Thr:	threonine
Trp:	tryptophan
t-BOC:	tert-butoxycarbonyl
Cbz:	benzyloxycarbonyl
DMF:	dimethylformamide
THF:	tetrahydrofuran
HPLC:	high-performance liquid chromatography
TLC:	thin-layer chromatography
UV:	ultraviolet

The A-21978C antibiotics are closely related, acidic peptide antibiotics described in U.S. Patent No. 4,208,403, which is incorporated herein by reference. As described in U.S. Patent No. 4,208,403, the A-21978 antibiotic complex contains a major component, factor C, which is itself a complex of closely related factors. A-21978 factor C, which is called the A-21978C complex, contains individual factors C_0, C_1, C_2, C_3, C_4 and C_5. Factors C_1, C_2 and C_3 are major factors; and factors C_0, C_4 and C_5 are minor factors. The structure of the A-21978C factors is shown in formula 3:



3

in which 3MG represents L-threo-3-methylglutamic acid, and R^N represents a specific fatty acid moiety. The specific R^N groups of the factors are as follows:

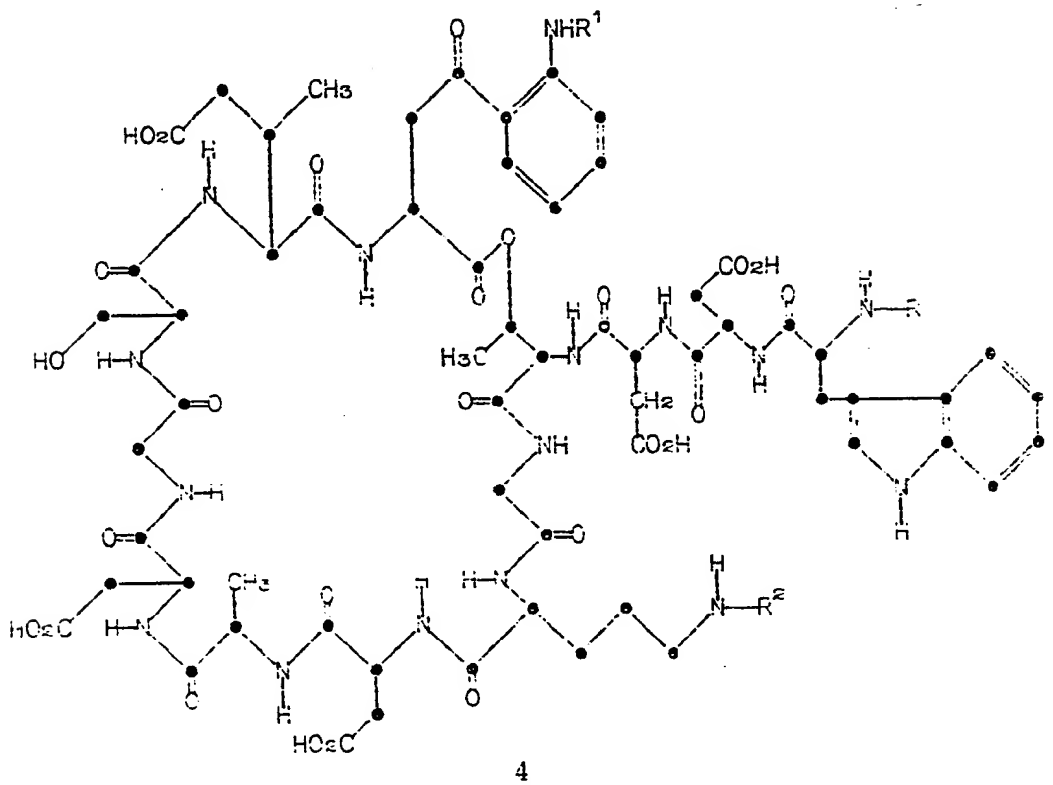
A-21978C Factor	R^N Moiety
C_1	8-methyldecanoyl
C_2	10-methylundecanoyl
C_3	10-methyldodecanoyl
C_0	C_{10}-alkanoyl*
C_4	C_{12}-alkanoyl*
C_5	C_{12}-alkanoyl*

*Identity not yet determined

It is extremely difficult, when confronted with the problem of deacylating a peptide antibiotic to know whether an enzyme exists which can be used for this purpose. Finding such an enzyme is even more difficult when the substrate antibiotic contains a cyclic peptide nucleus. Because enzymes have a high degree of specificity, differences in the peptide moiety and in the side chain of the substrate will affect the outcome of the deacylation attempt. In addition, many microorganisms make a large number of peptidases which attack different portions of the peptide moiety. This frequently leads to intractable mixtures of products.

In each of the A-21978C antibiotics (formula 3), the fatty acid side chain (R^N) is attached at the α-amino group of the tryptophan residue. We have discovered that the fatty acid side chain can be cleaved by an enzyme without affecting the chemical integrity of the remainder of the A-21978C peptide.

The present invention relates also to a novel process for obtaining A-21978C cyclic peptide nuclei having formula 4



4

in which R is selected from the group consisting of hydrogen, an amino-protecting group, 8-methyldecanoyl, 10-methylundecanoyl, 10-methyldodecanoyl, the specific C_{10}-alkanoyl group of A-21978C factor C_0 and the specific C_{12}-alkanoyl moieties of A-21978C factors C_4 and C_5; R^1 and R^2 are, independently, hydrogen or an amino-protecting group; provided that, when R is other than hydrogen or an amino-protecting group, at least one of

R^1 and R^2 must be an amino-protecting group; or to a pharmaceutically-acceptable salt of these compounds. The cyclic peptides of formula 4 or their pharmaceutically-acceptable salts are useful intermediates in the preparation of new semi-synthetic antibacterial agents which are especially useful against gram-positive microorganisms.

The term "amino-protecting group" as used throughout this description refers to a recognized amino-protecting group which is compatible with the other functional groups in the A-21978C molecule. Preferably, amino-protecting groups are those which can be readily removed subsequently. Examples of suitable protecting groups can be found in "Protective Groups in Organic Synthesis" by Theodora W. Greene, John Wiley and Sons, New York, 1981, Chapter 7. Especially preferred amino-protecting groups are the tert-butoxycarbonyl and benzyloxycarbonyl groups.

The cyclic peptides of formula 4 in which R is hydrogen and R^1 or R^2 is an amino-protecting group or hydrogen include the common cyclic peptide of A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5 (A-21978C nucleus) and blocked derivatives of A-21978C nucleus. A-21978C nucleus, i.e. the compound of formula 4 in which each of R, R^1 and R^2 represents hydrogen, is alternately described by formula 2.

A-21978C nucleus has the following characteristics:

Form: white amorphous solid which fluoresces under short-wave UV

Empirical formula: $C_{62}H_{82}N_{16}O_{26}$
Molecular weight: 1466
Solubility: soluble in water
Infrared absorption spectrum (KBr): shown in Figure 1 of the accompanying drawings; absorption maxima are observed at the following frequencies (cm^{-1}):

3300 (broad), 3042 (weak), 2909 (weak), 1655 (strong), 1530 (strong), 1451 (weak), 1399 (medium), 1222 (medium), 1165 (weak), 1063 (weak) and 758 (medium to weak)

Ultraviolet (UV) absorption spectrum (methanol): UV maxima 223 nm (ε 41,482) and 260 nm (ε 8,687)

Electrometric titration (66% aqueous dimethylformamide): indicates the presence of four titratable groups with pK_a values of about 5.2, 6.7, 8.5 and 11.1 (initial pH 6.12)

A particularly useful cyclic peptide of formula 4 is the compound in which R and R^1 represent hydrogen and R^2 represents tert-butoxycarbonyl. For convenience, this compound is designated "t-BOC nucleus". A-21978C t-BOC nucleus has the following characteristics:

Form: white amorphous solid which fluoresces under short-wave UV

Empirical formula: $C_{67}H_{90}N_{16}O_{28}$
Molecular weight: 1566
Solubility: soluble in water
Infrared absorption spectrum (KBr): shown in Figure 2 of the accompanying drawings; absorption maxima are observed at the following frequencies (cm^{-1}):

3345 (broad), 3065 (weak), 2975 (weak), 2936 (weak), ∿1710 (shoulder), 1660 (strong), 1530 (strong), 1452 (weak), 1395 (medium), 1368 (weak), 1341 (weak), 1250 (medium), 1228 (medium), 1166 (medium to weak) and 1063 (weak)

Ultraviolet absorption spectrum (90% ethanol): UV maxima 220 nm (ε 42,000) and 260 nm (ε 10,600)

High-performance liquid chromatography: retention time = 6 min on 4.6- x 300-mm silica-gel C_{18} column, using $H_2O/CH_3CN/CH_3OH$ (80:15:5) solvent containing 0.2% NH_4OAc at a flow rate of 2 ml/min with UV detection.

The blocked A-21978C factors of this invention are those compounds of formula 1 in which at least one of R^1 and R^2 is an amino-protecting group and R is selected from 8-methyldecanoyl, 10-methylundecanoyl, 10-methyldodecanoyl, the specific C_{10}-alkanoyl group of A-21978C factor C_0 and the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5.

The A-21978C blocked factors and cyclic peptides of this invention are capable of forming salts which are also part of this invention. Such salts are useful, for example, for separating and purifying the compounds. Pharmaceutically-acceptable alkali-metal, alkaline-earth-metal, amine and acid-addition salts are particularly useful. "Pharmaceutically-acceptable" salts are salts which are useful in the chemotherapy of warm-blooded animals.

For example, the A-21978C cyclic peptides of formula 1 have five free carboxyl groups which can form

salts. Partial, mixed and complete salts of these carboxyl groups are contemplated, therefore, as part of this invention. In preparing these salts, pH levels greater than 10 should be avoided due to the instability of the compounds at such levels.

Representative and suitable alkali-metal and alkaline-earth metal salts of the A-21978C cyclic peptides of formula 1 include, among others, the sodium, potassium, lithium, cesium, rubidium, barium, calcium and magnesium salts. Suitable amine salts of the A-21978C cyclic peptides include the ammonium and the primary, secondary, and tertiary C_1-C_4-alkylammonium and hydroxy-C_2-C_4-alkylammonium salts. Illustrative amine salts include, among others, those formed by reaction of an A-21978C cyclic peptide with ammonium hydroxide, methylamine, sec-butylamine, isopropylamine, diethylamine, di-isopropylamine, cyclohexylamine, ethanolamine, triethylamine, and 3-amino-1-propanol.

The alkali-metal and alkaline-earth-metal cationic salts of the A-21978C cyclic peptides of formula 1 are prepared according to procedures commonly used for the preparation of cationic salts. For example, the free acid form of the A-21978C cyclic peptide is dissolved in a suitable solvent such as warm methanol or ethanol; a solution containing the stoichiometric quantity of the desired inorganic base in aqueous methanol then is added. The salt thus formed can be isolated by routine methods, such as filtration or evaporation of the solvent. Another convenient method of preparing salts is by the use of ion-exchange resins.

The salts formed with organic amines can be prepared in a similar manner. For example, the gaseous or liquid amine can be added to a solution of an A-21978C cyclic peptide in a suitable solvent such as acetone; the solvent and excess amine can be removed by evaporation.

The A-21978C cyclic peptides of this invention also have free amino groups and can form, therefore, acid addition salts which are also part of this invention. Representative and suitable acid-addition salts of the A-21978C cyclic peptides include, among others, those salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, sulfuric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, D-glutamic, d-camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, or cinnamic acids.

Preparation of the A-21978C Cyclic Peptides

The A-21978C cyclic peptides of formula 1 in which R is hydrogen and R^1 or R^2 is an amino-protecting group or hydrogen are obtained by deacylating a peptide antibiotic selected from the group consisting of A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5 and blocked A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5.

I. Preparation of the Substrates

A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5 are prepared as described in U.S. Patent No. 4,208,403. These factors are components of the A-21978C complex which is part of the A-21978 complex.

The A-21978 complex may be produced by culturing Streptomyces roseosporus NRRL 11379 (deposited August 29, 1978) under submerged aerobic fermentation conditions until a substantial level of antibiotic activity is produced. The A-21978 complex is separated by filtering the fermentation broth, lowering the pH of the filtrate to about pH 3, allowing the complex to precipitate, and separating the complex by filtration. The separated complex may be purified further by extraction techniques. For isolation of the individual A-21978C complex and factors, chromatographic separations are required.

Blocked A-21978C complex and the blocked A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5 of this invention (compounds of formula 1 in which R is selected from the group consisting of 8-methyldecanoyl, 10-methylundecanoyl, 10-methyldodecanoyl, and the C_{10}- and C_{12}-alkanoyl groups of factors C_0, C_4 and C_5) are prepared using procedures for protecting amino groups in peptides. The protecting groups are selected from the various known amino-protecting groups such as, for example, benzyloxycarbonyl, t-butoxycarbonyl, t-amyloxycarbonyl, isobornyloxycarbonyl, adamantyloxycarbonyl, o-nitrophenylthio, diphenylphosphinothioyl, chloro- or nitrobenzyloxycarbonyl.

Blocked A-21978C complex is especially advantageous as a substrate. It is prepared from the A-21978C complex, thereby avoiding the separation steps required to obtain the individual A-21978C factors; but, when it is deacylated, it gives a single product, i.e. the appropriately blocked nucleus.

II. The Deacylation Procedure

A. Preparation of the Enzyme

1. The Producing Microorganisms

The enzymes which are useful for deacylation of A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5 and blocked factors C_0, C_1, C_2, C_3, C_4 and C_5 may be produced by certain microorganisms of the family Actinoplanaceae, preferably the microorganism Actinoplanes utahensis NRRL 12052 (deposited October 9, 1979). Although a preferred method of cultivating A. utahensis NRRL 12052 to produce this enzyme is described in Example 1, those skilled in the art will recognize that other methods may be used.

The Actinoplanaceae are a family of microorganisms of the order Actinomycetales. First described by Dr. John N. Couch, this family was established in 1955 [J. Elisha Mitchell Sci. Soc. 71, 148-155 (1955)]. The characteristics of the family and of many individual genera are found in "Bergey's Manual of Determinative Bacteriology", 8th ed., R. E. Buchanan and N. E. Gibbons, Eds., The Williams & Wilkins Co., Baltimore, Md., 1974, pages 706-723. Ten genera have

thus far been distinguished: I. Actinoplanes (the type genus and thus far the most common genus); II. Spirillospora; III. Streptosporangium; IV. Amorphosporangium; V. Ampullariella; VI. Pilimelia; VII. Planomonospora; VIII. Planobispora; IX. Dactylosporangium; and X. Kitasatoa.

Some of the species and varieties which have been isolated and characterized so far are: Actinoplanes philippinensis, Actinoplanes armeniacus, Actinoplanes utahensis, and Actinoplanes missouriensis; Spirillospora albida; Streptosporiangium roseum, Streptosporangium vulgare, Streptosporangium roseum var. hollandensis, Streptosporangium album, Streptosporangium viridialbum, Amorphosporangium auranticolor, Ampullariella regularis, Ampullariella campanulata, Ampullariella lobata, Ampullariella digitata, Pilimelia terevasa, Pilimelia anulata, Planomonospora parontospora, Planomonospora venezuelensis, Planobispora longispora, Planobispora rosea, Dactylosporangium aurantiacum, and Dactylosporangium thailandense.

The genus Actinoplanes is a preferred source of the enzyme which is useful for this invention. Within the genus Actinoplanes, the species Actinoplanes utahensis is an especially preferred source.

Cultures of other representative useful species which produce the enzyme are available to the public from the Northern Regional Research Center, Agricultural Research Culture Collection (NRRL), U.S. Department of Agriculture, 1815 North University St., Peoria, Illinois 61604, U.S.A., under the following accession numbers:

Actinoplanes utahensis	NRRL 12052 (deposited October 9, 1979)
Actinoplanes missouriensis	NRRL 12053 (deposited October 9, 1979)
Actinoplanes sp.	NRRL 8122 (deposited Ocrober 17, 1975)
Actinoplanes sp.	NRRL 12065 (deposited November 5, 1979)
Streptosporangium roseum var. hollandensis	NRRL 12064 (deposited November 5, 1979)

A. utahensis NRRL 12052 was derived from a parent culture which was also deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852 (A. utahensis ATCC 14539). The A. utahensis ATCC 14539 culture may also be used as a source of the enzyme.

A. missouriensis NRRL 12053 was derived from a culture which was also deposited with ATCC (A. missouriensis ATCC 14538) and which is another source of the enzyme.

The effectiveness of any given strain of microorganism within the family Actinoplanaceae for carrying out the deacylation of this invention is determined by the following procedure. A suitable growth medium is inoculated with the microorganism. The culture is incubated at about 30°C. for two or three days on a rotary shaker. One of the substrate antibiotics is added then to the culture maintaining the pH of the fermentation medium at about pH 7.0. The culture is monitored for activity using a Micrococcus luteus assay. This procedure is described in Sect. D.

Loss of antibiotic activity is an indication that the microorganism produces the requisite enzyme for deacylation. This must be verified, however, using one of the following methods: 1) analysis by HPLC for presence of the intact nucleus; or 2) re-acylation with an appropriate side chain (e.g. lauroyl, n-decanoyl or n-dodecanoyl) to restore activity. Reduction in antibiotic activity is difficult to distinguish when deacylating blocked A-21978C material because addition of the blocking group causes an 80-90% reduction in antibiotic activity.

2. Conditions for Enzyme Production

Production of the enzyme occurs under conditions satisfactory for growth of the Actinoplanaceae, i.e., a temperature between about 25 and about 30°C. and a pH of between about 5.0 and about 8.0, with agitation and aeration. The culture medium should contain a) an assimilable carbon source such as sucrose, glucose, glycerol, or the like; b) a nitrogen source such as peptone, urea, ammonium sulfate, or the like; c) a phosphate source such as a soluble phosphate salt; and d) inorganic salts found generally to be effective in promoting the growth of microorganisms. An effective amount of the enzyme is generally obtained in from about 40 to about 60 hours after the beginning of the growth cycle and persists for some time after the effective growth has been reached. The amount of enzyme produced varies from species to species of the organism and in response to different growth conditions.

As will be apparent, the microorganisms which produce the enzyme, such as Actinoplanes utahensis NRRL 12052, are subject to variation. For example, artificial variants and mutants of these strains may be obtained by treatment with various known mutagens such as ultraviolet rays, X-rays, high-frequency waves, radioactive rays, and chemicals. All natural and artificial variants and mutants which are obtained from the Actinoplanaceae and which produce the enzyme may be used in this invention.

B. Deacylation Conditions

The substrate used as the starting material preferably is added to the culture of Actinoplanaceae after the culture has been incubated for about 48 hours. The concentration of substrate in the conversion medium can vary widely. For maximum use of the enzyme and for substantially complete deacylation within a three-hour period, however, the concentration of substrate will generally range from about one to about three mg/ml. Lower concentrations can be used, but may not make maximum use of the enzyme; higher concentrations can be used also, but the substrate may not be completely deacylated unless the fermentation time is extended.

Conversion of the substrate antibiotic to A-21978C nucleus according to this invention proceeds best when the pH of the fermentation medium is maintained in the range of from about 7.0 to about 7.2. Below pH 7, deacylation proceeds slowly; as pH values

move above pH 7.2, the nucleus which is formed is increasingly subject to alkaline hydrolysis. In stirred fermentors the pH may be controlled by sensor controllers. Where this is impractical, such as in flask fermentors, pH may be controlled by adding 0.1 molar phosphate buffer to the medium prior to addition of the substrate.

After addition of the substrate, incubation of the culture should be continued for about 3-6 hours or longer. The purity of the substrate will affect the rate of deacylation. For example, a substrate having a purity of greater than 50 percent is deacylated at a rate of about 2.5 mg/ml of antibiotic in 3 hours. When substrates of lower purity are used, the deacylation proceeds at a somewhat slower rate.

Multiple substrate feedings may be made. For example, 0.75 mg/ml of antibiotic may be fed at 24-hour intervals for at least five or more additions.

The deacylation can be carried out over a broad temperature range, e.g. from about 20 to about 45°C. It is preferable, however, to carry out the deacylation at a temperature of about 30°C. for optimum deacylation and stability of substrate and nucleus.

C. The Substrate

It is preferable, but not essential, to use purified antibiotic as the substrate. Because purified substrate is soluble in water or in buffer, it can be handled more conveniently. Moreover, with purified substrate the deacylation proceeds more rapidly.

Semipurified substrates containing as little as 15 percent of the starting antibiotic have been deacylated successfully.

The substrate antibiotics have antibacterial activity. Thus, although the substrate materials (especially those of low purity) may harbor bacterial cells or spores which presumably could grow in the deacylation medium and affect the deacylation reaction or the stability of the starting antibiotic or the product nucleus, this has not been observed. It is not necessary, therefore, that the substrates be sterile, especially for short deacylation periods.

D. Monitoring the Deacylation

A-21978C factors C_0, C_1, C_2, C_3, C_4 and C_5 are antibacterial agents which are especially active against Micrococcus luteus. For this reason an assay using M. luteus is preferred for determining quantities of substrate present. The A-21978C nucleus which is formed is water soluble, but is biologically inactive. Reduction in biological activity is, therefore, a quick, presumptive test for deacylation.

The amount of nucleus formed can be quantitated by HPLC analysis, using the system described.

E. Use of Resting Cells

An alternate method of deacylation involves removing the Actinoplanaceae cells from the culture medium, resuspending the cells in a buffer solution, and carrying out the deacylation as described in Sect. B. When this method is used, the enzymatically active

mycelia can be reused. For example, A. utahensis NRRL 12052 mycelia retain deacylase activity after storage for one month or longer under refrigeration (4-8° C.) or in the frozen state (-20° C.). A preferred buffer solution is 0.1 molar phosphate buffer.

F. Immobilized Enzymes

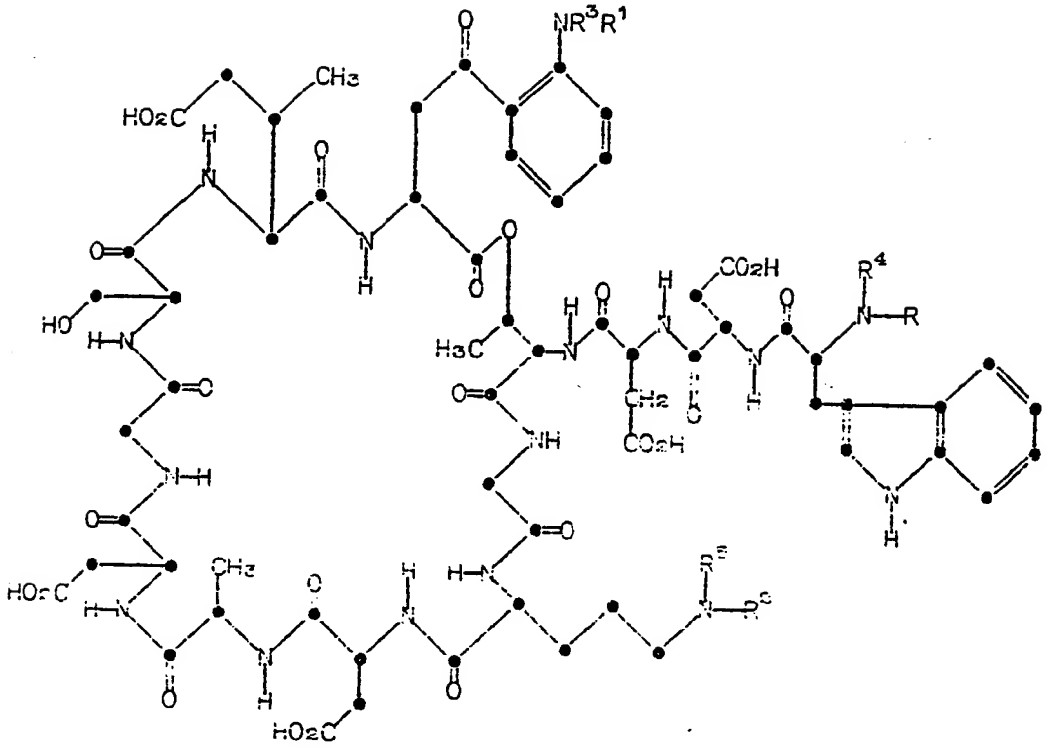
Yet another method of carrying cut the deacylation is to immobilize the enzyme by methods known in the art. (See, for example, "Biomedical Applications of Immobilized Enzymes and Proteins", Thomas Ming Swi Chang, Ed., Plenum Press, New York, 1977; Vol. 1.) The immobilized enzyme can then be used in a column (or other suitable type of reactor) to effect the deacylation.

In addition, the microorganism itself can be immobilized and used to catalyze the deacylation reaction.

Utility of the A-21978C Cyclic Peptides

The A-21978C cyclic peptide nuclei and their salts are useful intermediates in the preparation of semi-synthetic antibacterial compounds.

Therefore, in another aspect of this invention, compounds having the structure shown in formula 1:



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in which R, R^1 and R^2 are, independently, hydrogen, C_4-C_{14}-alkyl, optionally substituted C_2-C_{19}-alkanoyl, C_5-C_{19}-alkenoyl or an amino-protecting group, R^3, R^4 and R^5 are all hydrogen, or (i) R^3 and R^1; and/or (ii) R^4 and R, and/or (iii) R^5 and R^2, taken together may represent a C_4-C_{14} alkylidene group, provided that 1) the R, R^1 and R^2 groups must together contain at least four carbon atoms, and 2) when R^1 and R^2 are both selected from hydrogen group, R cannot be 8-methyldecanoyl, 10-methylundecanoyl, 10-methyldodecanoyl, the specific C_{10}-alkanoyl group of A-21978C factor C_0 or

the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5; or a pharmaceutically-acceptable salt thereof, are chemotherapeutically-useful antibiotics.

The term "C_4-C_{14}-alkylidenyl" refers to a group of the formula $\begin{matrix} R^{11} \\ \diagdown \\ R^{12}\diagup C= \end{matrix}$ in which R^{11} and R^{12} are hydrogen or an alkyl group of from 3 to 13 carbon atoms, provided that one of R^{11} and R^{12} must be other than hydrogen and further provided that the sum of the carbon atoms in R^{11} and R^{12} must be no greater than 13. Those compounds in which at least one of R, R^1 or R^2 is C_4-C_{14}-alkylidenyl are known as Schiff's bases.

The term "C_4-C_{14}-alkyl" refers to a univalent saturated, straight- or branched-chain alkyl group containing from 4 to 14 carbon atoms. Those compounds in which one of R, R^1 or R^2 are C_4-C_{14}-alkyl are prepared by reduction of the corresponding compounds where the R, R^1 or R^2 group is C_4-C_{14}-alkylidenyl. The resulting alkyl derivatives are referred to as "reduced Schiff's bases".

The compounds of formula 1 in which one or more of the amino groups are substituted by an alkylidenyl (the Schiff's bases) or alkyl group (the reduced Schiff's bases) can be prepared by known methods for preparing Schiff's bases and reducing such bases, respectively. Thus, the Schiff's bases are prepared by reaction (condensation) of the primary amino group of tryptophan, ornithine or kynurenine of A-21978C with an appropriate aldehyde or ketone in a suitable solvent. Reduction of

the imine bond of the Schiff's base, to obtain the corresponding formula I compound in which R, R^1 or R^2 is alkyl, can be accomplished by known selective reduction procedures. A preferred reducing agent for this reaction is sodium cyanoborohydride.

Under the in vitro test conditions used, the Schiff's bases do not show antibacterial activity, possibly due to their instability in the assay medium. They are useful, however, as intermediates to the reduced Schiff's bases. When the Schiff's base is used as an intermediate, it is not necessary to isolate the intermediate prior to reducing it to form the reduced Schiff's base.

The terms "optionally substituted C_2-C_{19}-alkanoyl" and "C_5-C_{19}-alkenoyl" refer to acyl groups derived from carboxylic acids containing from 2 to 19 and 5 to 19 carbon atoms, respectively. When the R group is alkanoyl, the alkyl portion is a univalent saturated, straight-chain or branched-chain hydrocarbon radical which optionally can bear one hydroxyl group or from one to three halo substituents selected from chlorine, bromide, and fluorine. When R is alkenoyl, the alkenyl portion is a univalent, unsaturated, straight-chain or branched-chain hydrocarbon radical containing not more than three double bonds. Any one of the double bond(s) of the unsaturated hydrocarbon chain may be in the cis or trans configuration.

The term "amino-protecting group" refers to a recognized amino-protecting group as defined supra.

The following are preferred embodiments of these compounds of formula 1:

(a) The compounds in which R is alkanoyl of the formula $CH_3(CH_2)_n-\overset{O}{\overset{\|}{C}}-$, in which n is an integer from 3 to 17;

(b) The compounds in which R is alkanoyl of the formula $CH_3(CH_2)_n-\overset{O}{\overset{\|}{C}}-$, in which n is 5 to 14;

(c) The compounds in which R is alkanoyl of the formula $CH_3(CH_2)_n\overset{CH_3}{\overset{|}{C}}H(CH_2)_m-\overset{O}{\overset{\|}{C}}-$, in which n and m are each, independently, an integer from 0 to 14, provided that n + m must be no less than 1 and no greater than 15; and further provided that, if R^1 and R^2 are hydrogen, when n is 0, m cannot be 8 and, when n is 1, m cannot be 6 or 8;

(d) The compounds in which R is cis or trans alkenyl of the formula

$$CH_3(CH_2)_nCH=CH(CH_2)_m-\overset{O}{\overset{\|}{C}}-$$

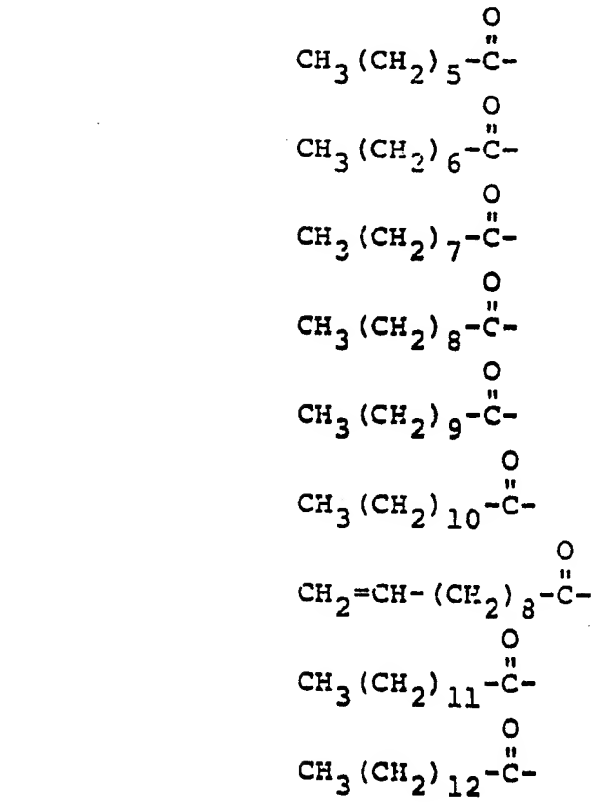
in which n and m are each, independently, an integer from 0 to 14, provided that n + m must be no less than 1 and no greater than 15;

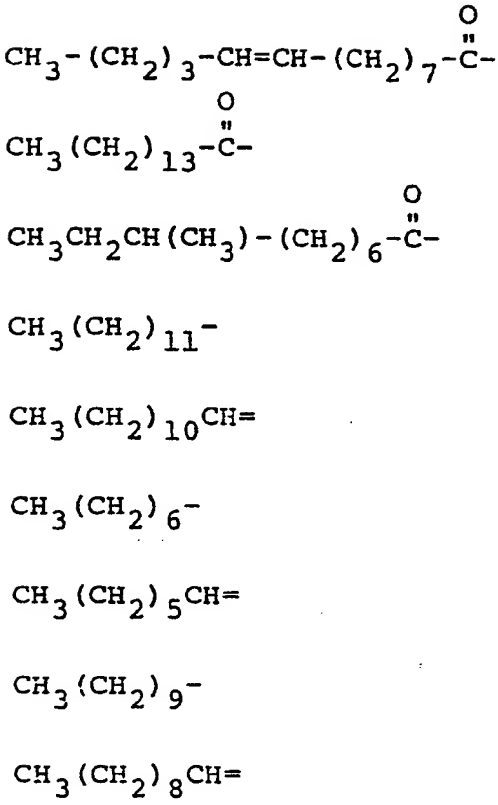
(e) The compounds in which R is cis or trans alkenyl of the formula

in which n is an integer from 4 to 15;

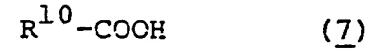
(f) The compounds in which R is alkyl of the formula $CH_3(CH_2)_n$- and n is an integer from 5 to 12; and

(g) The compounds in which R is:



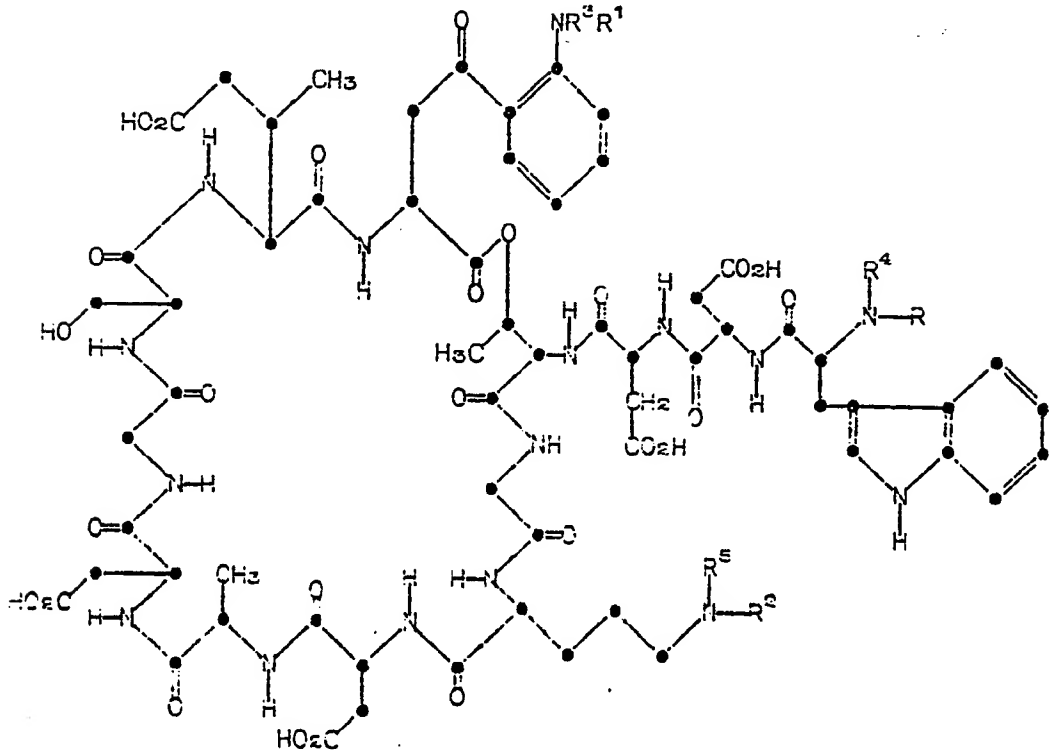


These alkanoyl and alkenoyl derivatives of formula 1 are prepared by acylating an A-21978C nucleus with the desired alkanoyl or alkenoyl side chain using conventional methods for forming an amide bond. In general, the acylation is accomplished by reacting a nucleus with an activated derivative of the acid (formula 7) corresponding to the desired acyl side chain.



(R^{10} is optionally substituted C_1-C_{18} alkyl or C_4-C_{18} alkenyl).

In another aspect of this invention, other chemotherapeutically-useful compounds have the general formula 1:



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These additional derivatives are those in which R is hydrogen, 8-methyldecanoyl, 10-methyldodecanoyl, 10-methylundecanoyl, the specific C_{10}-alkanoyl group of A-21978C_0 or the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5, an amino-protecting group, an aminoacyl group of the formula $-\overset{O}{\overset{\|}{C}}-Q-NH_2$ in which Q is C_1-C_{16} alkylene or an

N-alkanoylaminoacyl group of the formula $-W-\overset{O}{\overset{\|}{C}}-R^7$ in which:

W is a divalent aminoacyl radical of the formula:

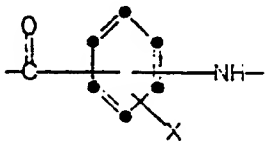
(a) $-\overset{O}{\overset{\|}{C}}-A-NH-$

in which A is C_1-C_{10} alkylene or C_5-C_6 cycloalkylene;

(b) $-\overset{O}{\overset{\|}{C}}-\overset{R^8}{\overset{|}{C}}H-NH-$

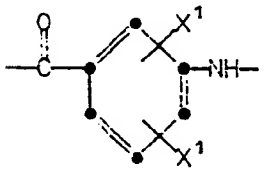
in which R^8 is hydroxymethyl, hydroxyethyl, mercaptomethyl, mercaptoethyl, methylthioethyl, 2-thienyl, 3-indole-methyl, phenyl, benzyl, or substituted phenyl or substituted benzyl in which the benzene ring thereof is substituted with chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

(c)

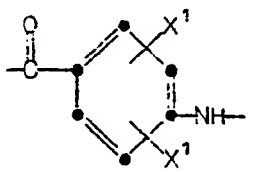


in which X is hydrogen, chloro, bromo, iodo, amino, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy, mercapto, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

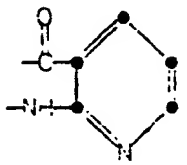
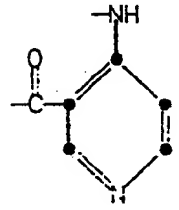
(d)

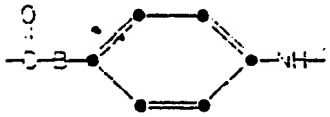


or



in which X^1 is chloro, bromo, iodo, amino, hydroxy, C_1-C_3-alkyl or C_1-C_3-alkoxy;

(e)  or  ; or

(f) 

in which B is a divalent radical of the formula: $-(CH_2)_n-$ and n is an integer from 1 to 3; $-CH=CH-$; $-CH=CH-CH_2-$; or $-CH_2NHC-$ (with O); R^7 is C_1-C_{17} alkyl or C_2-C_{17} alkenyl; R^1, R^3, R^4 and R^5 are hydrogen and R^2 is hydrogen, an amino-protecting group, an aminoacyl group of the formula $-C(=O)-Q-NH_2$ as herein defined, or an N-alkanoylaminoacyl group of the formula $-W-C(=O)-R^7$ as herein defined; provided that when R is other than hydrogen, aminoacyl, an amino-protecting group or N-alkanoylaminoacyl, R^2 must be aminoacyl, N-alkanoylaminoacyl or an amino-protecting group; or a pharmaceutically-acceptable salt thereof.

The terms "alkylene", "alkyl", "alkoxy", "alkylthio", and "alkenyl" as used here comprehend both straight and branched hydrocarbon chains. "Alkyl" means a univalent saturated hydrocarbon radical.

"Alkenyl" means a univalent unsaturated hydrocarbon radical containing one, two, or three double bonds, each oriented in the cis or trans configuration. "Alkylene" means a divalent saturated hydrocarbon radical. "Cycloalkylene" means a divalent cyclic saturated hydrocarbon radical.

Illustrative C_1-C_{10} or C_1-C_{16} alkylene radicals which are preferred are:

$-CH_2-$; $-\overset{R^5}{\underset{|}{C}}H-$ in which R^5 is C_1-C_4 alkyl (i.e., methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, i-butyl, or 1-methylpropyl); $-(CH_2)_m-$ in which m is an integer from 2 to 10; and $CH_3-(CH_2)_q-\overset{|}{C}H-(CH_2)_p-$, in which p is an integer from 1 to 8 and q is an integer from 0 to 7, provided that p + q must be no greater than 8.

For this aspect of the invention, illustrative C_1-C_{17} alkyl groups which are preferred are:

(a) CH_3-;

(b) $-(CH_2)_nCH_3$ in which n is an integer from 1 to 16; and

(c) $-(CH_2)_r\overset{CH_3}{\underset{|}{C}}H(CH_2)_sCH_3$ in which r and s are, independently, an integer from 0 to 14 provided that r + s can be no greater than 14.

Illustrative C_2-C_{17} alkenyl radicals which are preferred are:

(a) $-(CH_2)_t-CH=CH-(CH_2)_u-CH_3$ in which t and u are, independently, an integer from 0 to 14 provided that t + u can be no greater than 14.

(b) $-(CH_2)_v-CH=CH-(CH_2)_y-CH=CH-(CH_2)_z-CH_3$ in which v and z are, independently, an integer from 0 to 11 and y is an integer from 1 to 12 provided that v + y + z can be no greater than 12.

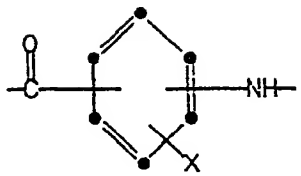
In particular, the following embodiments of the C_1-C_{17} alkyl groups are preferred:

CH_3-
$CH_3(CH_2)_5-$
$CH_3(CH_2)_6-$
$CH_3(CH_2)_8-$
$CH_3(CH_2)_{10}-$
$CH_3(CH_2)_{12}-$
$CH_3(CH_2)_{14}-$
$CH_3(CH_2)_{16}-$

In particular, the following embodiments of the C_2-C_{17} alkenyl groups are preferred:

cis-$CH_3(CH_2)_5CH=CH(CH_2)_7-$
trans-$CH_3(CH_2)_5CH=CH(CH_2)_7-$
cis-$CH_3(CH_2)_{10}CH=CH(CH_2)_4-$
trans-$CH_3(CH_2)_{10}CH=CH(CH_2)_4-$
cis-$CH_3(CH_2)_7CH=CH(CH_2)_7-$
trans-$CH_3(CH_2)_7CH=CH(CH_2)_7-$
cis-$CH_3(CH_2)_5CH=CH(CH_2)_9-$
trans-$CH_3(CH_2)_5CH=CH(CH_2)_9-$
cis, cis-$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7-$
trans, trans-$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7-$
cis,cis,cis-$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH-(CH_2)_7-$.

When "W" is a divalent radical of the formula



it will be recognized by those skilled in the art that the $-\overset{O}{\overset{\|}{C}}-$ function and the -NH- function may be oriented on the benzene ring in the ortho, meta, or para configuration relative to each other. The substituent represented by X may be substituted at any available position of the benzene ring. Preferred embodiments are those in which X is hydrogen and the $-\overset{O}{\overset{\|}{C}}-$ and -NH- functions are oriented in the para configuration.

The terms "substituted phenyl" and "substituted benzyl", as defined in connection with R^8, contemplate substitution of a group at any of the available positions in the benzene ring--i.e. the substituent may be in the ortho, meta, or para configuration. The term "C_1-C_3 alkyl" as defined in connection with R^8 or X includes the methyl, ethyl, n-propyl, or i-propyl groups.

Illustrative R and/or R^2 aminoacyl and N-alkanoylaminoacyl groups are provided in the Examples, infra. Other such illustrative R and/or R^2 groups are:

4-[N-(n-octanoyl)amino]cyclohexan-1-carbonyl,
7-[N-(n-heptanoyl)amino)-n-octanoyl,

α-hydroxymethyl-α-[N-(n-pentadecanoyl)amino]-acetyl,

α-(m-methoxyphenyl)-α-[N-(n-heptanoyl)amino]-acetyl,

m-chloro-p-[N-(n-nonanoyl)amino]benzoyl,

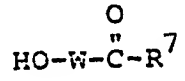
2,4-dihydroxy-5-[N-(n-decanoyl)amino]benzoyl,

4-[N-(3-methylbutanoyl)amino]nicotinoyl,

4-[N-(n-heptadecanoyl)amino]phenylpropionyl and

p-[N-(n-hexadecanoyl)amino]hippuryl.

These compounds of formula 1 are prepared by acylating an A-21978C nucleus with the appropriate N-alkanoylaminoacyl or N-alkenoylaminoacyl side chain using methods conventional in the art for forming an amide bond. The acylation is accomplished, in general, by reacting a nucleus with an activated derivative of the acid (formula 5) corresponding to the desired acyl side chain group.



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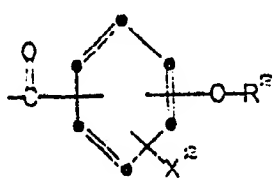
(W and R^7 have the meaning described herein supra).

In yet another aspect of this invention, other derivatives of structure 1 are those in which R is a substituted benzoyl group of the formula



in which X^2 is hydrogen, chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkylthio; R^1, R^3, R^4 and R^5 are hydrogen; R^2 is hydrogen or an amino-protecting group; and R^6 is C_8-C_{15} alkyl; or a pharmaceutically-acceptable salt thereof.

The substituted benzoyl group, the $-\overset{O}{\overset{\|}{C}}-$ function and the $-OR^6$ function may be oriented on the benzene ring in the ortho, meta, or para position relative to each other. The para orientation for these groups is preferred. The substituent represented by X^2 may be substituted at any available position of the benzene ring not occupied by these two groups.

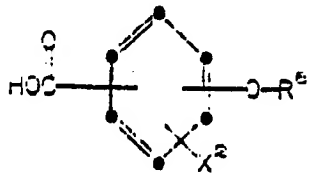
The term "alkyl" as used in this aspect of the invention comprehends both straight and branched hydrocarbon chains.

Illustrative C_8-C_{15} alkyl radicals which are preferred for R^6 are:

(a) $-(CH_2)_nCH_3$ in which n is an integer from 7 to 14; and

(b) $-(CH_2)_r\overset{CH_3}{\overset{|}{C}}H(CH_2)_sCH_3$ in which r and s are, independently, an integer from 0 to 12, provided that r + s can be no greater than 12 or no less than 5.

These compounds of formula 1 are prepared by acylating an A-21978C nucleus, using methods conventional in the art for forming an amide bond. The acylation is accomplished, in general, by reacting the selected compound with an activated derivative of the substituted benzoic acid (formula 6) corresponding to the desired acyl group (R, R^1, R^2):



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(X^2 and R^6 have the meanings described supra).

Illustrative compounds of formula 6 include the following: p-(n-octyloxy)benzoic acid, p-(n-decyloxy)benzoic acid, p-(n-dodecyloxy)benzoic acid, p-(n-pentadecyloxy)benzoic acid, m-chloro-p-(n-dodecyloxy)benzoic acid, p-chloro-m-(n-decyloxy)benzoic acid, m-(n-dodecyloxy)-p-methylbenzoic acid, m-methoxy-p-(n-octyloxy)benzoic acid and m-hydroxy-p-(n-pentyloxy)benzoic acid.

"Activated derivative", as used throughout the specification, means a derivative which renders the carboxyl function of the acylating agent reactive to coupling with a primary amino group to form the amide bond which links the acyl side chain to the nucleus. Suitable activated derivatives, their methods of preparation, and their use as acylating agents for a primary

amine will be recognized by those skilled in the art. Preferred activated derivatives are: (a) an acid halide (e.g. an acid chloride), (b) an acid anhydride (e.g. an alkoxyformic acid anhydride or aryloxyformic acid anhydride) or (c) an activated ester (e.g. a 2,4,5-trichlorophenyl ester, an N-hydroxy-benztriazole ester, or an N-hydroxysuccinimide ester). Other methods for activating the carboxyl function include reaction of the carboxylic acid with a carbonyldiimide (e.g. N,N'-dicyclohexylcarbodiimide or N,N'-diisopropyl-carbodiimide) to give a reactive intermediate which, because of instability, is not isolated. Such a reaction with the primary amine is carried out in situ.

Those skilled in the art will recognize that the compounds of formula 1 are prepared using selective acylation procedures with the assistance of amino-protecting groups. For example, when a compound of formula 1 in which R, R^1 and R^2 are hydrogen is the starting material, acylation can occur at both the α-amino group of tryptophan and the δ-amino group of ornithine to give N_{Trp}, N_{Orn}-diacyl derivatives. To obtain derivatives monoacylated at the α-amino group of tryptophan, therefore, it is preferable to acylate a compound of formula 1 in which the δ-amino group of ornithine (the R^2 position) is blocked by an amino-protecting group. Such starting materials are preferably obtained by blocking the A-21978C factor at this position before it is deacylated. The aromatic amino group of kynurenine is the least reactive of the three free amino groups in the A-21978C nucleus. Acylation

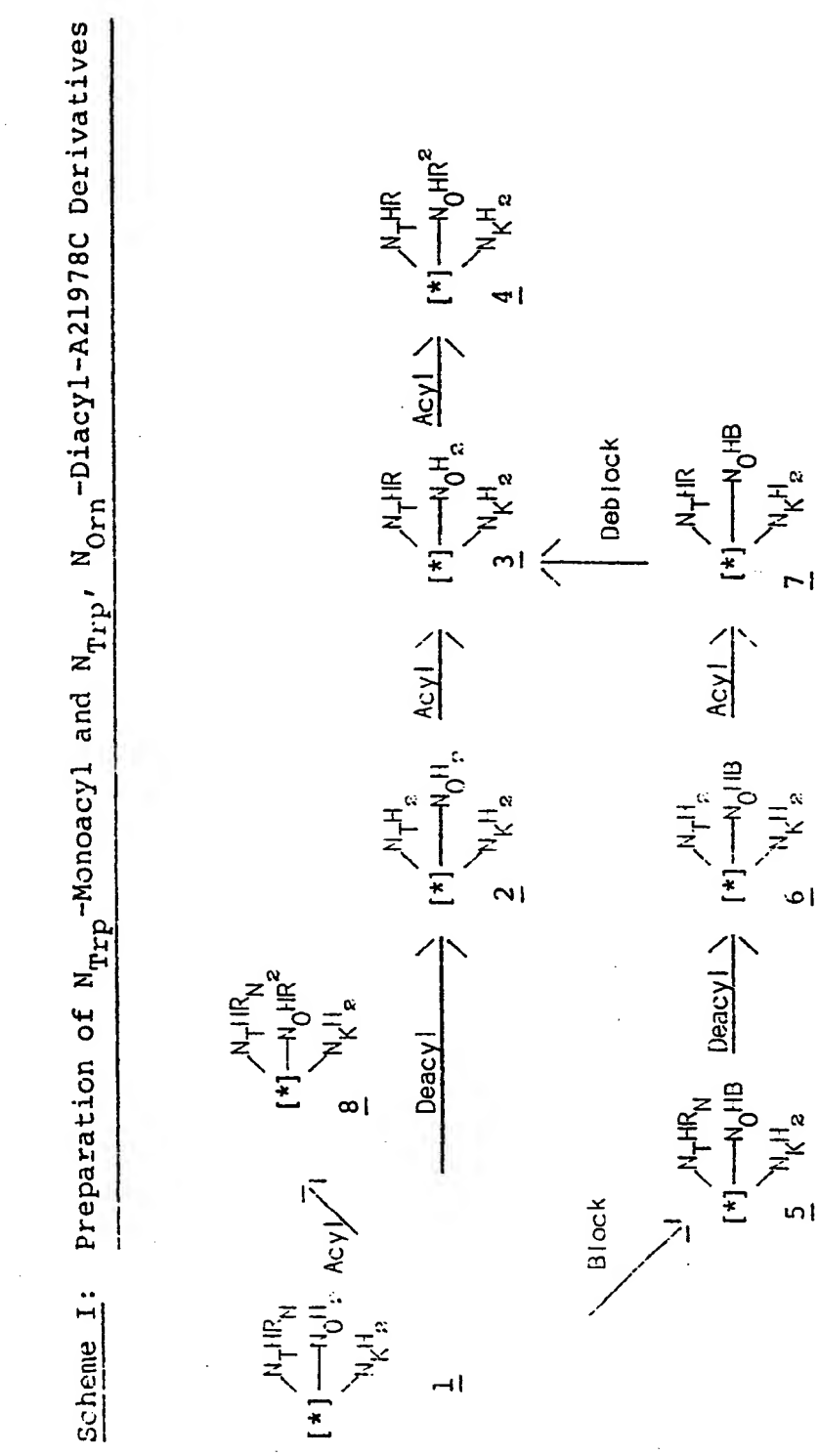
at kynurenine involves appropriate blocking of the amino groups of tryptophan and ornithine, but acylation at R or R^2 does not usually involve blocking the amino group of kynurenine.

Schemes I, II, and III outline general procedures for the preparation of the compounds of formula 1 in which one of R, R^1 or R^2 is alkanoyl, alkenoyl, or an amino-protecting group. In these Schemes the following symbols are used:

[*]	= remainder of A-21978C
N_T	= α-amino group of tryptophan
N_O	= δ-amino group of ornithine
N_K	= aromatic amino group of kynurenine
R, R^1, R^2	= substituents as defined
R_N	= acyl group of natural factor
B, B^1	= amino-protecting groups
Acyl	= an acylation step
Deacyl	= a deacylation step
Block	= acylation with an amino-protecting group
Deblock	= removal of an amino-protecting group

In Scheme I the N_{Trp}-monoacyl derivatives of A-21978C are represented by general formula 3 and the N_{Trp}, N_{Orn}-diacyl derivatives of A-21978C are represented by general formula 4. Those N_{Trp}, N_{Orn}-diacyl derivatives in which the N_{Trp}-acyl group is that of a natural A-21978C factor are represented by general formula 8.

Scheme I: Preparation of N_{Trp}-Monoacyl and N_{Trp}, N_{Orn}-Diacyl-A21978C Derivatives



In Scheme II the N_{Trp}, N_{Kyn}-diacyl derivatives of A-21978C are represented by general formula 10. Those N_{Trp}, N_{Kyn}-diacyl derivatives in which the N_{Trp}-acyl group is that of a natural A-21978C factor are represented by general formula 12. The compounds having general formulas 5, 6 and 7 are also described in Scheme I.

Scheme II: Preparation of N_{Trp}, N_{Kyn}-Diacyl-A-21978C Derivatives

In Scheme III the N_{Orn}-monoacyl derivatives of A-21978C are represented by general formula 18, the N_{Kyn}-monoacyl derivatives of A-21978C are represented by general formula 15, and the N_{Orn}, N_{Kyn}-diacyl derivatives of A-21978C are represented by general formula 20. The compounds having general formula 6 are described also in Schemes I and II.

Scheme III: Preparation of N_{Orn}-Monoacyl, N_{Kyn}-Monoacyl and N_{Orn},N_{Kyn}-Diacyl-A-21978C Derivatives

A preferred method for preparing the compounds of formula 1 is the active ester method. The 2,4,5-trichlorophenyl ester of the desired acid is a preferred acylating agent. In this method, an excess amount of the active ester is reacted with a formula 1 compound at room temperature in a non-reactive organic solvent such as DMF, THF, diethyl ether or dichloromethane. The reaction time is not critical, although a time of about 6 to about 120 hours is preferred. At the conclusion of the reaction, the solvent is removed, and the residue is purified by a recognized method, such as by column chromatography. t-BOC groups can be removed by treatment with trifluoroacetic acid/anisole/triethylsilane or, preferably, trifluoroacetic acid/1,2-ethanedithiol for from about three to about five minutes at room temperature. After the solvent is removed, the residue can be purified by reversed-phase HPLC.

The 2,4,5-trichlorophenyl esters of the corresponding acids can be prepared conveniently by treating the desired acid with 2,4,5-trichlorophenol in the presence of a coupling agent, such as N,N'-dicyclohexylcarbodiimide. Other methods suitable for preparing acid esters will be apparent to those skilled in the art.

The alkanoic and alkenoic acids used in one aspect of this invention as starting materials and the activated derivatives thereof (in particular, the acid chlorides and the 2,4,5-trichlorophenyl esters), are known compounds or can be prepared from known compounds by known methods. The 2,4,5-trichlorophenyl

esters are made conveniently by treating the acid chloride of the alkanoic or alkenoic acid with 2,4,5-trichlorophenol in the presence of pyridine or by treating the free alkanoic or alkenoic acid with 2,4,5-trichlorophenol in the presence of N,N'-dicyclohexylcarbodiimide. The 2,4,5-trichlorophenyl ester derivative can be purified by column chromatography over silica gel.

An alternative acylation method for preparing the alkanoyl and alkenoyl derivatives is a modified Schotten-Baumann procedure in which the unblocked nucleus is treated with the acid chloride of the desired alkanoic acid or alkenoic acid in a pyridine/water mixture. In this method, an excess of the acid chloride in a non-reactive organic solvent (such as acetone) is added slowly to a solution of the nucleus in 90% pyridine/10% water (by volume). The unreacted acid chloride is separated from the reaction product by extraction into an immiscible organic solvent (e.g., diethyl ether). Final purification is by reversed-phase HPLC, as previously described.

The N-alkanoylamino acids or N-alkenoylamino acids used as starting materials for derivatives of formula 1 are either known compounds or they can be made by acylating the appropriate amino acid with the desired alkanoyl or alkenoyl group using conventional methods. A preferred way of preparing the N-alkanoylamino acids is by treating the appropriate amino acid with an alkanoic acid chloride in pyridine. The alkanoic or alkenoic acids, the activated derivatives

thereof, and the amino acids used are either known compounds or they can be made by known methods or by modification of known methods apparent to those skilled in the art.

If a particular amino acid contains an acylable functional group other than the amino group, it will be understood by those skilled in the art that such a group must be protected prior to reaction of the amino acid with the reagent used to attach the N-alkanoyl or N-alkenoyl group. Suitable protecting groups can be any group known in the art to be useful for the protection of a side chain functional group in peptide synthesis. Such groups are well known, and the selection of a particular protecting group and its method of use will be readily known to one skilled in the art [see, for example, "Protective Groups In Organic Chemistry", M. McOmie, Editor, Plenum Press, N.Y., 1973].

Certain amino acids used in the synthesis of these products may exist in optically active forms. Both the natural configuration (L-configuration) and D-configuration may be used as starting materials.

The substituted benzoic acids used as starting materials for some of the A-21978C derivatives, and the activated derivatives thereof are either known compounds or they can be made from known compounds by methods known in the art. The alkoxybenzoic acids can be prepared conveniently from an appropriate hydroxybenzoic acid by reacting an appropriate alkyl halide with the disodium salt of the appropriate hydroxybenzoic acid.

The hydroxybenzoic acids and substituted derivatives thereof used as starting materials in the processes described are either known compounds or can be prepared by conventional methods.

The compounds prepared from an A-21978C nucleus inhibit the growth of pathogenic bacteria as evidenced by standard biological test procedures. The compounds are useful, therefore, for controlling the growth of bacteria on environmental surfaces (as an antiseptic) or in treating infections caused by bacteria. The antibiotic activity of the compounds has been demonstrated in vitro in agar-plate disc-diffusion tests and in agar-dilution tests and in vivo in tests in mice infected with Staphylococcus aureus and Streptococcus pyogenes. The compounds are particularly useful in treating infections caused by gram-positive organisms.

When an A-21978C cyclic peptide of this invention is used as an antibiotic, it may be administered either orally or parenterally. As will be appreciated by those skilled in the art, the A-21978C compound is commonly administered together with a pharmaceutically acceptable carrier or diluent.

The compounds can be administered intravenously or intramuscularly by injection in the form of a sterile aqueous solution or suspension to which may be added, if desired, various conventional pharmaceutically acceptable preserving, buffering, solubilizing, or suspending agents. Other additives, such as saline or glucose may be added to make the solutions isotonic. The proportions and nature of such additives will be apparent to those skilled in the art.

For oral use, the compounds can be administered in combination with pharmaceutically acceptable carriers or excipients in the form of capsules, tablets or powders. The nature and proportion of such carriers or excipients will be recognized by those skilled in the art.

The dosage of A-21978C compound will depend upon a variety of considerations, such as, for example, the particular compound being used and the nature and severity of the infection to be treated. Those skilled in the art will recognize that appropriate dosage ranges or dosage units for administration may be determined by considering the MIC and ED_{50} values and toxicity data provided together with factors such as pharmacokinetics, characteristics of the patient or host and the infecting microorganism.

In order to illustrate more fully the invention, the following non-limiting examples are provided.

EXAMPLE 1

Preparation of A-21978C Nucleus

A. Fermentation of Actinoplanes utahensis

A stock culture of Actinoplanes utahensis NRRL 12052 is prepared and maintained on an agar slant. The medium used to prepare the slant is selected from one of the following:

MEDIUM A

Ingredient	Amount
Pre-cooked oatmeal	60.0 g
Yeast	2.5 g
K_2HPO_4	1.0 g
Czapek's mineral stock*	5.0 ml
Agar	25.0 g
Deionized water	q.s. to 1 liter

pH before autoclaving is about 5.9; adjust to pH 7.2 by addition of NaOH; after autoclaving, pH is about 6.7.

*Czapek's mineral stock has the following composition:

Ingredient	Amount
$FeSO_4 \cdot 7H_2O$ (dissolved in 2 ml conc HCl)	2 g
KCl	100 g
$MgSO_4 \cdot 7H_2O$	100 g
Deionized water	q.s. to 1 liter

MEDIUM B

Ingredient	Amount
Potato dextrin	5.0 g
Yeast extract	0.5 g
Enzymatic hydrolysate of casein*	3.0 g
Beef extract	0.5 g
Glucose	12.5 g
Corn starch	5.0 g
Meat peptone	5.0 g
Blackstrap molasses	2.5 g
$MgSO_4 \cdot 7H_2O$	0.25 g
$CaCO_3$	1.0 g
Czapek's mineral stock	2.0 ml
Agar	20.0 g
Deionized water	q.s. to 1 liter

*N-Z-Amine A, Humko Sheffield Chemical, Lyndhurst, N.J.

The slant is inoculated with Actinoplanes utahensis NRRL 12052, and the inoculated slant is incubated at 30°C for about 8 to 10 days. About 1/2 of the slant growth is used to inoculate 50 ml of a vegetative medium having the following composition:

Ingredient	Amount
Pre-cooked oatmeal	20.0 g
Sucrose	20.0 g
Yeast	2.5 g
Distiller's Dried Grain*	5.0 g
K_2HPO_4	1.0 g
Czapek's mineral stock	5.0 ml
Deionized water	q.s. to 1 liter

adjust to pH 7.4 with NaOH; after autoclaving, pH is about 6.8.

*National Distillers Products Co., 99 Park Ave., New York, N.Y.

The inoculated vegetative medium is incubated in a 250-ml wide-mouth Erlenmeyer flask at 30°C for about 72 hours on a shaker rotating through an arc two inches in diameter at 250 RPM.

This incubated vegetative medium may be used directly to inoculate a second-stage vegetative medium. Alternatively and preferably, it can be stored for later use by maintaining the culture in the vapor phase of liquid nitrogen. The culture is prepared for such storage in multiple small vials as follows: In each vial is placed 2 ml of incubated vegetative medium and 2 ml of a glycerol-lactose solution [see W. A. Dailey and C. E. Higgens, "Preservation and Storage of Microorganisms in the Gas Phase of Liquid Nitrogen, Cryobiol 10, 364-367 (1973) for details]. The prepared suspensions are stored in the vapor phase of liquid nitrogen.

A stored suspension (1 ml) thus prepared is used to inoculate 50 ml of a first-stage vegetative medium (having the composition earlier described). The inoculated first-stage vegetative medium is incubated as above-described.

In order to provide a larger volume of inoculum, 10 ml of the incubated first-stage vegetative medium is used to inoculate 400 ml of a second-stage vegetative medium having the same composition as the first-stage vegetative medium. The second-stage medium is incubated in a two-liter wide-mouth Erlenmeyer flask at 30°C for about 48 hours on a shaker rotating through an arc two inches in diameter at 250 RPM.

Incubated second-stage vegetative medium (80 ml), prepared as above-described, is used to inoculate 10 liters of sterile production medium selected from one of the following:

MEDIUM I

Ingredient	Amount (g/L)
Peanut meal	10.0
Soluble meat peptone	5.0
Sucrose	20.0
KH_2PO_4	0.5
K_2HPO_4	1.2
$MgSO_4 \cdot 7H_2O$	0.25
Tap water	q.s. to 1 liter

The pH of the medium is about 6.9 after sterilization by autoclaving at 121°C for 45 minutes at about 16-18 psi.

MEDIUM II

Ingredient	Amount (g/L)
Sucrose	30.0
Peptone	5.0
K_2HPO_4	1.0
KCl	0.5
$MgSO_4 \cdot 7H_2O$	0.5
$FeSO_4 \cdot 7H_2O$	0.002
Deionized water	q.s. to 1 liter

Adjust to pH 7.0 with HCl; after autoclaving, pH is about 7.0.

MEDIUM III

Ingredient	Amount (g/L)
Glucose	20.0
NH_4Cl	3.0
Na_2SO_4	2.0
$ZnCl_2$	0.019
$MgCl_2 \cdot 6H_2O$	0.304
$FeCl_3 \cdot 6H_2O$	0.062
$MnCl_2 \cdot 4H_2O$	0.035
$CuCl_2 \cdot 2H_2O$	0.005
$CaCO_3$	6.0
KH_2PO_4*	0.67
Tap water	q.s. to 1 liter

*Sterilized separately and added aseptically

Final pH about 6.6.

The inoculated production medium is allowed to ferment in a 14-liter fermentation vessel at a temperature of about 30°C for about 66 hours. The fermentation medium is stirred with conventional agitators at about 600 RPM and aerated with sterile air to maintain the dissolved oxygen level above 30% of air saturation at atmospheric pressure.

B. Deacylation of A-21978C

A fermentation of A. utahensis is carried out as described in Section A, using slant medium A and production medium I and incubating the production medium for about 67 hours. Crude A-21978C complex, prepared as described in U.S. Patent No. 4,208,403, (100 g) is added to the fermentation medium.

Deacylation of the A-21978C complex is monitored by assay against Micrococcus luteus. The fermentation is allowed to continue until deacylation is complete as indicated by disappearance of activity vs. M. luteus, a period of about 24 hours.

This same procedure was used to determine whether other microorganisms would produce the desired A-21978C nucleus. In particular, Actinoplanes missouriensis NRRL 12053, Actinoplanes sp. NRRL 8122, Actinoplanes sp. NRRL 12065, and Streptosporangium roseum var. hollandensis NRRL 12064, when used in place of Actinoplanes utahensis NRRL 12052 in the procedure above, were found to produce the desired nucleus. HPLC comparisons using authentic samples of the nucleus obtained from this procedure using A. utahensis as the deacylating enzyme confirmed that these other microorganisms produced the A-21978C nucleus.

C. Isolation of A-21978C Nucleus

Whole fermentation broth (20 liters), obtained as described in Section B, was filtered with a filter aid (Hyflo Super-Cel, Johns Manville Corp.). The mycelial cake was discarded. The filtrate thus obtained was passed through a column containing 1.5 liters of HP-20 resin (DIAION High Porous Polymer, HP-Series, Mitsubishi Chemical Industries Limited, Tokyo, Japan). The effluent thus obtained was discarded. The column was then washed with deionized water (10 L.) to remove residual filtered broth. This wash water was discarded. The column was then eluted with water:acetonitrile mixtures (10 L. each of 95:5, 9:1, and 4:1), collecting 1-liter fractions.

Elution was monitored by analytical HPLC, using silica gel/C_{18} and a solvent system of water:-methanol (3:1) containing 0.1% ammonium acetate, detecting the nucleus with a UV monitor at 254 nm. Elution can be monitored also by paper chromatography, using an n-butane:pyridine:acetic acid:water (15:10:3:12) solvent system and detecting the compounds by UV fluorescence. In this system, the A-21978C factors have an R_f value of about 0.56 and A-21978C nucleus has an R_f of about 0.32. Fractions containing the nucleus were combined, concentrated under vacuum to remove the acetonitrile and freeze-dried to give 40.6 g of semi-purified A-21978C nucleus.

D. Purification of A-21978C Nucleus

Semi-purified A-21978C nucleus (15 g), obtained as described in Section C, was dissolved in 75 ml of water:methanol:acetonitrile (82:10:8) containing 0.2% acetic acid and 0.8% pyridine. This solution was pumped onto a 4.7- x 192-cm column containing 3.33 L. of silica gel (Quantum LP-1)/C_{18}. The column was developed with the same solvent system. Fractions having a volume of 350 ml were collected. Separation was monitored at 280 nm with a UV monitor. Fractions containing the nucleus were combined, concentrated under vacuum to remove solvents and freeze-dried to give 5.2 g of purified A-21978C nucleus.

EXAMPLE 2

Alternate Preparation of A-21978C Nucleus

A-21978C nucleus was prepared according to the method of Example 1 except for certain changes in Section B. The A. utahensis culture was incubated initially for about 48 hours; the substrate was semi-purified A-21978C complex (50 g); and incubation after addition of the substrate was about 16 hours. The broth filtrate was passed over a column containing 3.1 liters of HP-20 resin. The column was washed with 10 volumes of water and then was eluted with water:acetonitrile (95:5). Elution was monitored as in Example 1. After collecting 24 liters, the eluting solvent was changed to water:acetonitrile (9:1). Fractions containing the nucleus were eluted with this solvent.

These fractions were combined, concentrated under vacuum to remove acetonitrile, and freeze-dried to give 24.3 g of semi-purified A-21978C nucleus.

This semi-purified A-21978C nucleus (24.3 g) was dissolved in water (400 ml). The solution was pumped onto a 4.7- x 192-cm steel column containing 3.33 liters of silica gel (Quantum LP-1)/C_{18} prepared in water:methanol:acetonitrile (8:1:1) containing 0.2% acetic acid and 0.8% pyridine. The column was developed with the same solvent at a pressure of about 2000 psi, collecting 350 ml fractions. Elution was monitored by UV at 280 nm. Fractions containing the nucleus were combined, concentrated under vacuum to remove solvents, and freeze-dried to give 14 g of highly purified A-21978C nucleus.

EXAMPLE 3

Preparation of N_{Orn}-t-BOC A-21978C Factors C_2 and C_3

A mixture of A-21978C factors C_2 and C_3 (10 g), prepared as described in U.S. Patent No. 4,208,403, was dissolved in water (50 ml) with sonication (200 mg/ml). The pH of the solution was adjusted from 4.05 to 9.5 with 5N NaOH (3.6 ml). Di-tert-butyl dicarbonate (3.0 ml) was added, and the reaction mixture was stirred at room temperature for 2 hours. The pH of the reaction was maintained at 9.5 by manual addition of 5N NaOH (6.5 ml added in 2 hours).

The reaction was monitored periodically by TLC on silica gel, using $CH_3CN:H_2O$ (7:3 and 8:2) solvent systems and detecting by UV.

After about 10 minutes the reaction solution became rapidly turbid ,and base consumption increased. After 30 minutes, the rate of increase in turbidity and the rate of base consumption decreased, indicating that the reaction was complete. Nevertheless, the reaction was continued for an additional 90 minutes to insure completion. At the end of the two-hour reaction, the reaction material was lyophilized immediately to give 12.7 g of N_{Orn}-t-BOC-A-21978 factors C_2 and C_3.

Using similar procedures, two 10-g reactions and a 30-g reaction were run. In each of these the reaction time was only 40 minutes. The two 10-g experiments gave 11.9 and 12.1 g of product, respectively. The 30-g reaction gave 35.4 g of product.

EXAMPLE 4

Preparation of A-21978C N_{Orn}-t-BOC Nucleus

A. Fermentation of A. utahensis

A fermentation of A. utahensis was carried out as described in Example 1, Section A, using slant medium A and production medium I and incubating the production medium for about 66 hours.

B. Deacylation of N_{Orn}-t-BOC Complex

The A-21978C N_{Orn}-t-BOC complex (1185 g of crude substrate which contained about 176 g of A-21978C complex) was added to the fermentation medium. Deacylation was carried out as described in Example 1, Section B. Deacylation was complete, as indicated by HPLC, after about 24 hours.

C. Isolation of A-21978C N_{Orn}-t-BOC Nucleus

Fermentation broth (100 L.), obtained as described in Section B, was filtered with a filter aid (Hyflo Super-cel). The filtrate was passed over a column containing 7.5 L. of HP-20 resin (DIAION); the column was washed with water (38 L.). Elution was monitored by silica gel/C_{18} HPLC with UV detection at 254 nm. Some nucleus was eluted in the wash. Subsequent elution of nucleus was carried out with water: acetonitrile mixtures as follows: (95:5)-40 L.; (9:1)-40 L.; and (85:15)-100 L. Fractions containing the nucleus were combined, concentrated under vacuum to remove solvent, and freeze-dried to give 298.5 g of semi-purified A-21978C N_{Orn}-t-BOC nucleus.

D. Purification of A-21978C N_{Orn}-t-BOC Nucleus

Semi-purified A-21978C N_{Orn}-t-BOC nucleus (30 g), obtained as described in Section C, was dissolved in water:acetonitrile (9:1) containing 0.2% acetic acid and 0.8% pyridine (75 ml). This solution was applied to a 4.7 x 192-cm steel column containing 3.33 L. of silica gel (Quantum LP-1)/C_{18} equilibrated in the same solvent system. The column was developed under pressure with water:acetonitrile:methanol (80:15:5) containing 0.2% acetic acid and 0.8% pyridine, collecting 350-ml fractions and detecting product by UV at 280 nm. Fractions containing the product were combined, concentrated under vacuum to remove solvent and freeze-dried to give 18.4 g of purified A-21978C N_{Orn}-t-BOC nucleus.

EXAMPLE 5

Alternative Purification of A-21978C N_{Orn}-t-BOC Nucleus

Semi-purified A-21978C N_{Orn}-t-BOC nucleus (10.8 g), obtained as described in Example 4, Section C, was dissolved in water and applied to a column containing 80 ml of Amberlite IRA-68 (acetate cycle). The column was washed with water and, at a flow rate of 5 ml/min, was eluted sequentially with 0.05 N acetic acid (1080 ml), 0.1 N acetic acid (840 ml), and 0.2 N acetic acid (3120 ml), collecting 120-ml fractions. The column was monitored with analytical HPLC over silica gel/C_{18}, using a system of water:acetonitrile:methanol (80:15:5) containing 0.2% ammonium acetate and detecting product with UV at 254 nm. Fractions containing the product were combined; the pH of the solution was adjusted to 5.8 with pyridine; the resulting solution was concentrated under vacuum to a volume of about 200 ml. Water was added to the concentrate, and the resulting solution was reconcentrated to remove pyridine. This concentrate was freeze-dried to give 3.46 g of purified A-21978C N_{Orn}-t-BOC nucleus.

EXAMPLES 6-21

The preparation of various alkanoyl and alkenoyl derivatives by acylation of the A-21978C nucleus or blocked derivatives thereof, representative of the preparation of the compounds of formula 1, is shown in Table I, below. The derivatives in Table I are made either by the modified Schotten-Bauman reaction

using an acid chloride as the acylating agent (Method A) or by the active ester method using the 2,4,5-trichlorophenyl ester as the acylating agent (Method B). The general procedures for carrying out the acylation reactions by Method A or Method B are set forth below:

Method A (Modified Schotten-Bauman Reaction)

This method involves reaction of a A-21978C nucleus with the alkanoic or alkenoic acid chloride that corresponds to the desired acyl side chain.

An A-21978C nucleus or blocked derivative thereof (1.95-2.16 g, 1.33-1.47 mmoles) or an A-21978C factor (409 mg, 0.25 mmole) is dissolved in 200 ml of pyridine/H_2O (9:1). The acylating agent (18-20 mmoles excess acyl chloride dissolved in 15 ml acetone) is added dropwise over 1-3 hours, and the reaction is stirred at ambient temperature for an additional 2-3 hours. The reaction mixture is concentrated to remove the acetone. The aqueous phase which remains is diluted to a volume of about 200 ml with water. The pH of this solution is adjusted to pH 3.0 to 3.5 with glacial acetic acid. This solution is washed 8 times with equal volumes of diethyl ether and then is lyophilized.

The crude acylated derivative is purified by reversed-phase HPLC as follows: The sample, dissolved in water or the eluant system (about 4-6.5 ml), is injected onto a 33- x 5/8-inch stainless-steel column, packed with LP_1/C_{18} support. The column is eluted with a solvent system consisting of H_2O:MeOH:CH_3CN:pyridine:-HOAC. Elution is performed at a pressure of about

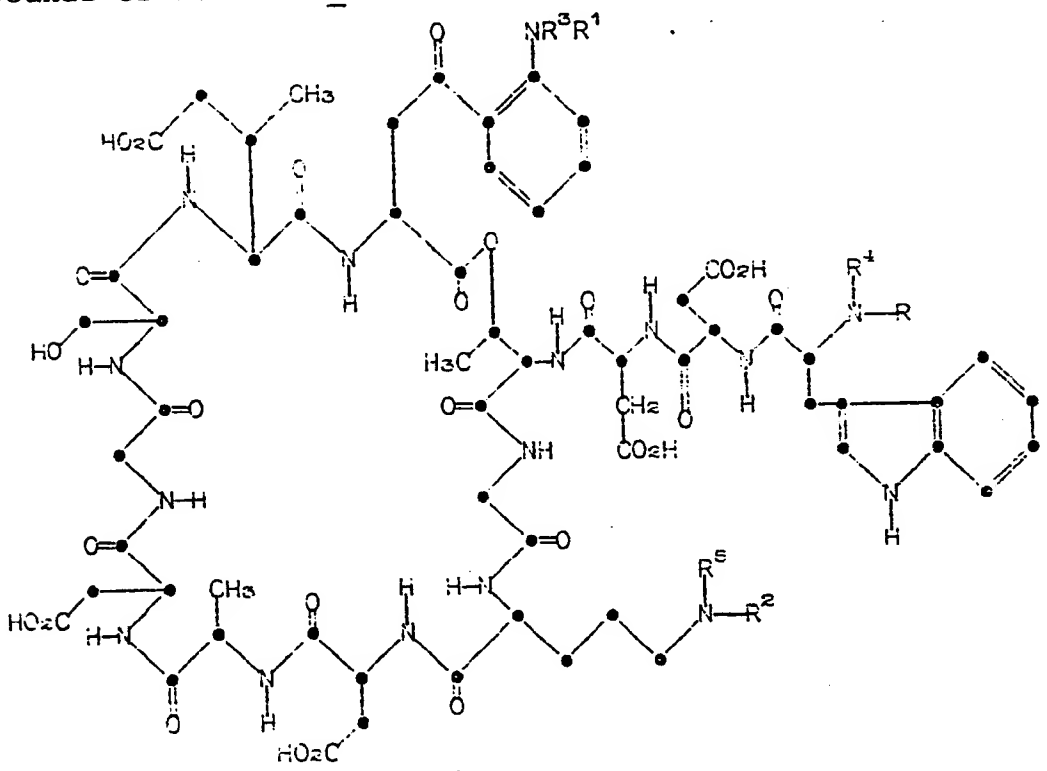
1500-2000 psi with a flow rate of about 10-12 ml/min, using an LDC duplex pump (Milton-Roy). Effluent is monitored by UV detection, using an ISCO-UA-5 detector at 280 nm. The desired fractions are combined and evaporated to dryness in vacuo to yield the desired alkanoyl derivative. The purified product is analyzed by TLC using reversed-phase plates (Whatman KC_{18}) and a H_2O:MeOH:CH_3CN:pyridine:HOAc (45:15:40:2:2) solvent system. The plates are observed under UV light to detect the product. The products are analyzed also by UV (extinction coefficients at 220 nm and 260 nm) and by amino acid analysis. Purity is determined by analytical reversed-phase HPLC (C_{18} Microbondapak, Waters Co.) with a H_2O:MeOH:CH_3CN:pyridine:HOAc solvent system, monitoring eluent with UV at 280 nm.

Method B (2,4,5-Trichlorophenyl Active Ester Method)

A solution of N_{Orn}-t-BOC A-21978C nucleus (1.0 g, 0.64 mmol), A-21978C nucleus (0.5-1.0 g, 0.34-0.68 mmole) or A-21978C, (946 mg, 0.58 mmole) and a 3.5 molar excess of trichlorophenyl-acyl active ester are dissolved in DMF (100 ml) and stirred at from about room temperature to about 50°C. for from about 6 to about 20 hours. The reaction mixture is concentrated to an oil in vacuo. The oil is triturated with 50 ml Et_2O:toluene (1:1) and washed with Et_2O. The mono-acylated and di-acylated A-21978C nucleus derivatives, acylated A-21978C_1, and acylated t-BOC A-21978C nucleus are purified as described in Method A.

The acylated t-BOC-A-21978C nucleus is deblocked using 50 mg/ml of trifluoroacetic acid:anisole:-triethylsilane (10:1:1) at from about -10° to about -0°C. for 3-5 minutes. This reaction mixture is concentrated in vacuo to an oil that is triturated with two 20-ml volumes of Et_2O. The crude acyl product is purified by reversed-phase HPLC and analyzed as described in Method A.

The specific compounds in the examples summarized in Tables 1 through 23 which follow are compounds of formula 1:



1

TABLE 1

Preparation of N_{Trp}-Monoacyl Derivatives of A-21978C Cyclic Peptides

Example No.	Compound[a] R	Method of Prep.	Nucleus wt (mg)	Acyl. Agent wt (mg)	Reaction Time (hr)	Intermediate[b] HPLC Eluent[c]	Product HPLC Eluent[c]	Product wt (mg)
6	$CH_3(CH_2)_5CO-$	B	1028	800	20	55:15:30	50:15:35	205
7	$CH_3(CH_2)_6CO-$	A	1950	3421	3	50:15:35	55:15:30	355
8	$CH_3(CH_2)_6CO-$	B	1000	350	24	50:15:35	115:15:64	264
9	$CH_3(CH_2)_7CO-$	A	2000	882	2	not purified	50:15:35	212
10	$CH_3(CH_2)_7CO-$	B	1000	400	20	not purified	50:15:35	240
11	$CH_3(CH_2)_8CO-$	A	2160	4595	5		45:15:40	334
12	$CH_3(CH_2)_9CO-$	B	1000	288	22	not purified	45:15:40	392
13	$CH_3(CH_2)_{10}CO-$	A	1000	3406	2		45:15:40	370
14	$CH_3(CH_2)_{11}CO-$	B	1000	800	24	10:15:75	40:15:45	313
15	$CH_3(CH_2)_{12}CO-$	B	1000	812	22[d]	50:15:35	1:0:1[e]	267
16	$CH_3(CH_2)_{12}CO-$	B	1000	900	20	50:15:35	50:15:35	215
17	$CH_3(CH_2)_{13}CO-$	B	1000	1031	22[d]	not purified	35:10:55[e]	337.8
18	$CH_3(CH_2)_{14}CO-$	B	1000	744	18[d]	not purified	2:0:3[e]	257
19	$CH_2=CH-(CH_2)_6CO-$	B	1000	237	20	20:15:65	45:15:40	290
20	$CH_3(CH_2)_3CH=CH(CH_2)_7CO-$	B	1000	400	48	not purified	40:15:45	215
21	$CH_3CH_2CH=CHCH_2CH=CH-CH_2CH=CH(CH_2)_7CO-$	B	1000	400	28	35:15:50	35:15:50	283

[a] R^1, R^2, R^3, R^4, R^5 = H, [b] N_{Trp}Acyl-N_{Orn}-tBOC, [c] H_2O:CH_3OH:CH_3CN(v:v:v) containing 0.2% pyridine and 0.2% HOAc

[d] Reaction temperature maintained at 5°C., [e] Containing 1% pyridine and 1% HOAc

TABLE 2

Characteristics of N_{Trp}-Monoacyl Derivatives of A-21978C Cyclic Peptides

Compound No.	R[d]	R^1	R^2	Product R_f[a]	Anal. HPLC Eluent[b]	UV $\varepsilon_{\lambda max}$ 220nm	UV $\varepsilon_{\lambda max}$ 260nm
1	$CH_3(CH_2)_5CO-$	H	H	0.85	60:15:25	48,100	11,400
2	$CH_3(CH_2)_6CO-$	H	H	0.80	60:15:25	46,900	10,500
3	$CH_3(CH_2)_7CO-$	H	H	0.75	45:15:40	46,000	11,000
4	$CH_3(CH_2)_8CO-$	H	H	0.70	45:15:40	46,500	10,000
5	$CH_3(CH_2)_9CO-$	H	H	0.65	45:15:40	46,200	10,400
6	$CH_3(CH_2)_{10}CO-$	H	H	0.59	45:15:40	44,000	9,500
7	$CH_3(CH_2)_{11}CO-$	H	H	0.53	45:15:40	48,500	9,800
8	$CH_3(CH_2)_{12}CO-$	H	H	0.42	50:0:49[c]	48,447	9,170
9	$CH_3(CH_2)_{13}CO-$	H	H	0.34	1:0:1[c]	50,172	9,751
10	$CH_3(CH_2)_{14}CO-$	H	H	0.25	39:0:60[c]	54,000	12,000
11	$CH_2=CH-(CH_2)_8CO-$	H	H	0.70	45:15:40	44,000	9,200
12	$CH_3(CH_2)_3CH=CH(CH_2)_7CO-$	H	H	0.54	45:15:40	45,000	9,600
13	$CH_3CH_2CH=CHCH_2CH=CH-CH_2CH=CH(CH_2)_7CO-$	H	H	0.50	45:15:40[c]	50,000	11,600

[a] R_f by reversed-phase silica-gel TLC (Whatman KC_{18} with fluorescent indicator) and $H_2O:CH_3OH:CH_3CN$ (45:15:40) with 0.2 % pyridine and 0.2% HOAc solvent system

[b] $H_2O:CH_3OH:CH_3N$(v:v:v) containing 0.2% pyridine and 0.2% HOAc

[c] Containing 1% pyridine and 1% HOAc

[d] R^3, R^4, R^5 = H

TABLE 3

Preparation of N_{Trp}-N_{Orn}-Diacyl A-21978C Derivatives

Example No.	R^f	R^1	R^2	Method of Prep.	Starting Material wt (mg)	Acyl. Agent wt (mg)	Reaction Time (hr)	HPLC Eluent[a]	Product Wt (mg)
22	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	CH_3CO-	A	409	2208	1.5	45:15:40	273
23	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	CH_3CO-	B	946	139	17	95:30:75	365
24	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	$HOCOCH_2CH_2CO-$	C[b]	500	62	20	45:15:40	201
25	$CH_3(CH_2)_6CO-$	H	$CH_3(CH_2)_6CO-$	B	500	530	18[c]	35:25:40	60
26	$CH_3(CH_2)_9CO-$	H	$CH_3(CH_2)_9CO-$	B	1000	292	24	45:15:40	158
27	$CH_3(CH_2)_9CO-$	H	$CH_3(CH_2)_9CO-$	B	1000	1119	18[c]	2:0:3[d]	366
28	$CH_3(CH_2)_{11}CO-$	H	$CH_3(CH_2)_{11}CO-$	B	1000	1066.9	18[c]	2:0:3[d]	245.9
29	$CH_3CH_2CH(CH_3)(CH_2)_6CO$	H	t-BOC	D[e]					
30	Cbz	H	$CH_3(CH_2)_{10}CO-$		1000	123	20		550

[a] H_2O:CH_3OH:CH_3N(v:v:v) containing 0.2% pyridine and 0.2% HOAc

[b] Succinic anhydride, 90% pyridine

[c] Reaction temperature maintained at 5°C.

[d] Containing 1% pyridine and 1% HOAc

[e] $(t\text{-BOC})_2/H_2O$

[f] R^3, R^4, R^5 = H

TABLE 4

Characteristics of N_{Trp}, N_{Orn}-Diacyl A-21978C Derivatives

Compound No.	R^e	R^1	R^2	Product R_f[a]	Product Anal. HPLC Eluent[b]	Product UV $\varepsilon_{\lambda max}$ 220nm	Product UV $\varepsilon_{\lambda max}$ 260nm
14	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	CH_3CO-	0.69	45:15:40	46,000	10,400
15	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	$HOCO(CH_2)_2CO-$	0.75	45:15:40	47,000	11,000
16	$CH_3(CH_2)_6CO-$	H	$CH_3(CH_2)_6CO-$	0.67	45:15:40[c]	45,000	9,000
17	$CH_3(CH_2)_9CO-$	H	$CH_3(CH_2)_9CO-$	0.31	30:15:55 45:15:40[c]	48,200	8,000
18	$CH_3(CH_2)_{11}CO-$	H	$CH_3(CH_2)_{11}CO-$	0.03	45:15:40[c]	49,000	9,000
19	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	t-BOC	NT[d]	NT	NT	NT
20	Cbz	H	$CH_3(CH_2)_{10}CO-$	0.58	NT	NT	NT

[a] R_f by reversed-phase silica-gel TLC (Whatman KC_{18}) with fluorescent indicator; solvent system H_2O:CH_3OH:CH_3CN (45:15:40) with 0.2% pyridine and 0.2% HOAc

[b] H_2O:CH_3OH:CH_3CN(v:v:v) containing 0.2% pyridine and 0.2% HOAc

[c] Containing 1% pyridine and 1% HOAc

[d] NT = not tested

[e] R^3, R^4, R^5 = H

TABLE 5

Preparation of N_{Trp}, N_{Kyn}-Diacyl Derivative[a]

Example No.	R,R^1	R^{2-5}	Method of Prep.	Starting Material wt (g)	Acyl Agent wt (g)	Reaction Time (hr)	tBOC Diacyl Intermediate HPLC Eluent[a]	Product HPLC Eluent[b]	Product wt (mg)
31	$CH_3(CH_2)_8CO-$	H	B	15	15	30	50:15:35	50:15:35	211

[a] See Example 33, infra

[b] $H_2O:CH_3OH:CH_3CN$(v:v:v) containing 0.2% pyridine and 0.2% HOAc

TABLE 6

Characteristics of N_{Trp}, N_{Kyn}-Diacyl Derivative

Compound No.	R	R^1	R^{2-5}	R_f[a]	Anal. HPLC Eluent[b]	UV $\epsilon_{\lambda max}$ 220nm	UV $\epsilon_{\lambda max}$ 260nm
21	$CH_3(CH_2)_8CO-$	$CH_3(CH_2)_8CO-$	H	0.66	61:15:23:1	41.800	11,500

[a] R_f by reversed-phase silica-gel TLC (Whatman KC_{18} with fluorescent indicator) and H_2O:CH_3OH:CH_3CN (45:15:40) with 0.2% pyridine and 0.2% HOAc solvent system

[b] H_2O:CH_3OH:CH_3CN:NH_4OAc containing 0.2% pyridine and 0.2% HOAc

EXAMPLE 32

N_{Trp}-(n-Decanoyl) A-21978C Nucleus (Compound 4)

The following procedure illustrates the large-scale preparation of compounds by the active-ester method.

A. Preparation of 2,4,5-Trichlorophenyl n-decanoate

A solution of decanoyl chloride (Pfaltz and Bauer, 5.6 ml) and 2,4,5-trichlorophenol (5.6 g) in diethyl ether (1 L) and pyridine (120 ml) is stirred for 4 hours. The reaction mixture is filtered and dried in vacuo. The 2,4,5-trichlorophenyl n-decanoate is purified on a silica-gel column (Woelm), using toluene as the eluent. Fractions are monitored by TLC, using short-wave UV for detection. Appropriate fractions are pooled and dried in vacuo to give 10.4 g of 2,4,5-trichlorophenyl n-decanoate.

B. Acylation of N_{Orn}-t-BOC-A-21978C Nucleus with 2,4,5-Trichlorophenyl n-decanoate

A solution of N_{Orn}-t-BOC A-21978C nucleus (15.0 g) and 2,4,5-trichlorophenyl n-decanoate (15.0 g) in dry DMF (500 ml) is stirred under N_2 at ambient temperature for 25 hours. The mixture is then stirred at 60°C. for 5 hours or until TLC shows reaction completion. The reaction mixture is concentrated in vacuo to about 200 ml and is stirred with 1.2 liters of Et_2O/toluene (5:1). The product is separated by filtration, washed with Et_2O, and dried under vacuum to give 15.05 g of the N_{Trp}-(n-decanoyl)-N_{Orn}-t-BOC A-21978C nucleus intermediate (formula 1; R=n-decanoyl, R^1=H, R^2=t-BOC).

C. Purification of N_{Trp}-(n-Decanoyl)-N_{Orn}-t-BOC-A-21978C Nucleus

The N_{Trp}-(n-decanoyl)-N_{Orn}-t-BOC-A-21978C nucleus intermediate is purified in the following manner: The crude preparation is dissolved in about 50 ml of the eluting solvent system, and then purified by HPLC, using the Waters Prep/500 system containing a cartridge packed with reversed-phase C_{18} silica-gel adsorbent. The system is eluted with H_2O:MeOH:CH_3CN (50:15:35) containing 0.2% pyridine and 0.2% HOAc. Fractions are monitored by UV at 280 nm. Appropriate fractions are combined and dried in vacuo to give 8.56 g of purified N_{Trp}-(n-decanoyl)-N_{Orn}-t-BOC-A-21978C nucleus.

D. Removal of the N_{Orn}-t-BOC Group

The t-BOC group is removed by stirring N_{Trp}-(n-decanoyl)-N_{Orn}-t-BOC A-21978C nucleus (1.47 g) in 15 ml of trifluoroacetic acid/1,2-ethanedithiol (10:1) at ambient temperature for 3 minutes. The reaction mixture is dried in vacuo, and the residue is triturated with Et_2O (50 ml). After a 20-ml Et_2O wash, the triturate is dried in vacuo to give 2.59 g of crude N_{Trp}-(n-decanoyl)-A-21978C nucleus (formula 1: R=n-decanoyl; R^1 and R^2=H).

E. Purification of N_{Trp}-(n-Decanoyl)-A-21978C Nucleus

The crude N_{Trp}-(n-decanoyl)-A-21978C nucleus is purified by reversed-phase HPLC in the following manner: The sample (2.59 g), dissolved in 4.0 ml of H_2O:MeOH:CH_3CN:pyridine:HOAc (50:15:35:2:2), is injected

onto a 33- x 1-inch stainless-steel column packed with LP-1/C_{18} adsorbent. The column is eluted with this same solvent system. Elution is performed at a pressure of 1200-1700 psi with a flow rate of 10-12 ml/min, using an LDC duplex pump (Milton-Roy). The effluent is monitored by a UV detector (Isco Model UA-5, Instrument Specialist Co., 4700 Superior Avenue, Lincoln, NB 68504) at 280 nm. Fractions (20-24 ml) are collected every two minutes. The desired fractions, as indicated by antimicrobial activity, are combined and dried *in vacuo* to give 1.05 g of product.

This purification procedure was repeated with 4.35 g, 4.25 g, 2.14 g, 2.00 g and 1.75 g crude starting derivative to give a total of 5.58 g of purified N_{Trp}-(*n*-decanoyl)-A-21978C nucleus.

EXAMPLE 33

N_{Trp}-(*n*-Decanoyl)-N_{Kyn}-(*n*-decanoyl)-A-21978C (Compound 21)

N_{Trp}-(*n*-Decanoyl)-N_{Kyn}-(*n*-decanoyl)-A-21978C nucleus (formula 1: R and R^1= *n*-decanoyl; R^2=H) is a minor reaction product in the preparation of the N_{Trp}-(*n*-decanoyl) derivative of Example 27. It is isolated during the reversed-phase HPLC purification described in Section E. Desired fractions are combined and dried *in vacuo* to give 211 mg of crude product. The compound is purified by analytical HPLC (C_{18} Microbondapak, Waters Co.), using H_2O:MeOH:CH_3CN:NH_4OH:HOAc (6:23:15:0.5:0.5) as the eluent system (32 repetitions, 500 µg each injected sample) to give 4.4 mg of N_{Trp}-(*n*-decanoyl)-N_{Kyn}-(*n*-decanoyl)-A-21978C nucleus, identified by 360 MHz proton NMR.

EXAMPLE 34

N_{Trp}-Cbz-N_{Orn}-Lauroyl A-21978C Nucleus (Compound 20)

This method involves protecting the tryptophan α-NH_2 of N_{Orn}-t-BOC-A-21978C nucleus with a Cbz group, then removing the t-BOC group and acylating (at the ornithine α-NH_2) with the trichlorophenyl-lauroyl ester.

A. Preparation of N_{Trp}-Cbz-N_{Orn}-t-BOC-A-21978C Nucleus

N_{Orn}-t-BOC-A-21978C nucleus (1.0 g) is dissolved in DMF (150 ml) and warmed to 60°C. N,O-bis-trimethylsilylacetamide (0.55 ml) is added, and then benzyl pentachlorophenyl carbonate (257 mg) is added. After being stirred for 20 hours, the reaction mixture is concentrated to a volume of about 20-25 ml. Water (100 ml) is added, and the pH of this solution is adjusted to 6.0 with 1N NaOH. The mixture is washed with Et_2O (6 times, 200-ml volumes) and then lyophilized.

The crude derivative is purified by reversed-phase HPLC as follows: The sample, dissolved in about 6 ml of H_2O:MeOH:CH_3CN:pyridine:HOAc (55:15:30:2:2), is injected onto a 33- x 1/2-inch stainless-steel column packed with LP-1/C_{18} support. The desired fractions are located by UV absorption (280 nm) and antimicrobial activity. These fractions are then combined and lyophilized to give 951 mg of the N_{Trp}-Cbz-N_{Orn}-t-BOC A-21978C nucleus derivative.

B. Removal of t-BOC Group

The t-BOC group is removed by dissolving the intermediate at 50 mg/ml in trifluoroacetic acid:-anisole:triethylsilane (10:1:1) at -10°C. for 3 minutes. The reaction is concentrated to an oil that is triturated with two 25-ml volumes of Et_2O to give 520 mg of crude N_{Trp}-Cbz-A-21978C nucleus.

C. Acylation of N_{Trp}-Cbz-A-21978C Nucleus

The N_{Trp}-Cbz-A-21978C nucleus is acylated by the trichlorophenyl active-ester acylation method. N_{Trp}-Cbz-A-21978C (520 mg) is added to a solution of 1-hydroxybenzotriazole (7 mg) and lauroyltrichlorophenol active ester (123 mg) in pyridine (150 ml). After being stirred for 20 hours at 60°C., the reaction mixture is concentrated to a residue that is triturated with Et_2O (3 times, 25-ml volumes) to give N_{Trp}-Cbz-N_{Orn}-lauroyl-A-21978C nucleus (550 mg).

EXAMPLE 35

Preparation of Schiff's Bases and Reduced Schiff's Bases

Several reactions were carried out as follows: A-21978C nucleus (40 mg) was dissolved in water (2 ml). The pH of the solution was adjusted from 4 to 9 with 1N NaOH. Aliquots (0.5 ml) of this solution were then mixed with each of the following aldehydes (5 µl):

1) heptaldehyde
2) octyl aldehyde
3) decyl aldehyde
4) undecyl aldehyde

in methanol (4 ml). The reactions were stirred overnight at room temperature. The Schiff's bases which formed were reduced with $NaBH_3CN$ (2.5 mg per reaction) for 5 minutes at room temperature.

The reactions were examined by silica-gel TLC, using a $CH_3CN:H_2O$ (7:3) solvent system, and by assay against Staphylococcus aureus. The Schiff's bases were not active against S. aureus in this test, perhaps due to their instability under the assay conditions. In each reduced reaction two factors were produced. These compounds were active against S. aureus and had a similar mobility in the TLC system, giving evidence that the following compounds of the formula I were produced (in each case R^1 is hydrogen):

Preparation of Schiff's Bases and Reduced Schiff's Bases

Reaction of A21978C with	Product R^b	Product R^2	Active vs S. aureus[a]
heptaldehyde	$CH_3(CH_2)_5CH=$	H	no
	$CH_3(CH_2)_6-$	H	yes
	$CH_3(CH_2)_5CH=$	$CH_3(CH_2)_5CH=$	no
	$CH_3(CH_2)_6-$	$CH_3(CH_2)_6-$	yes
octyl aldehyde	$CH_3(CH_2)_6CH=$	H	no
	$CH_3(CH_2)_7-$	H	yes
	$CH_3(CH_2)_6CH=$	$CH_3(CH_2)_6CH=$	no
	$CH_3(CH_2)_7-$	$CH_3(CH_2)_7-$	yes
decyl aldehyde	$CH_3(CH_2)_8CH=$	H	no
	$CH_3(CH_2)_9-$	H	yes
	$CH_3(CH_2)_8CH=$	$CH_3(CH_2)_8C=$	no
	$CH_3(CH_2)_9-$	$CH_3(CH_2)_9-$	yes
undecyl aldehyde	$CH_3(CH_2)_9CH=$	H	no
	$CH_3(CH_2)_{10}-$	H	yes
	$CH_3(CH_2)_9CH=$	$CH_3(CH_2)_9CH=$	no
	$CH_3(CH_2)_{10}-$	$CH_3(CH_2)_{10}-$	yes

[a] The lack of activity of the Schiff's bases in this test may reflect their instability under the test conditions.

[b] where R or R^2 is alkyl, then R^3, R^4 or R^5 = H.

EXAMPLE 36

Preparation of N_{Trp}-Lauraldehyde Schiff's Base and N_{Trp}, N_{Orn}-Di-Lauraldehyde Schiff's Base and Their Reduced Schiff's Bases (Compounds 22 and 23)

A-21978C nucleus (1 g) was dissolved in water (50 ml); a solution of dodecyl aldehyde (500 µl) in methanol (200 ml) was added. The reaction mixture was stirred overnight at room temperature. The reaction then was reduced for 50 minutes by the addition of $NaBH_3CN$ (291 mg). The reaction mixture was filtered under vacuum, using Whatman No. 1 paper to remove particulates. The supernatant was concentrated to an aqueous solution which was lyophilized to give 1.256 g of product. This product was evaluated by analytical HPLC and purified by preparative reverse-phase HPLC in portions. Each portion (350 mg) was dissolved in 50% aqueous methanol (6 ml), sonicating and heating to dissolve the material. The solution then was passed over a 1.5- x 80-cm LP_1-C_{18} column, eluting with CH_3OH:CH_3CN:H_2O (25:40:35) containing 0.2% pyridine and 0.2% acetic acid at a flow rate of about 7.5 ml/min. Elution was monitored by UV at 280 nm. Fractions containing the desired products were combined to give a total of 104 mg of N_{Trp}-(n-dodecyl)-A-21978C nucleus (Compound 22) and 19.2 mg of N_{Trp}-(n-dodecyl)-N_{Orn}-(n-dodecyl)-A-21978C nucleus (Compound 23).

EXAMPLE 37

The antibacterial activity of the compounds of formula 1 can be demonstrated in vitro. The results of the antibacterial testing of representative compounds of formula 1 using standard agar-plate disc-diffusion tests are set forth in Table 7. In Table 7 activity is measured by the size (diameter in mm) of the observed zone in which growth of the microorganism is inhibited by the test compound.

TABLE 7

Antibacterial Activity of Formula I Compounds by the Agar-Plate Disc-Diffusion Test

Compound No.	R^d	R^1	R^2	Size of Zone of Inhibition (mm)[a] Staphylococcus aureus ATCC 6738P	Bacillus subtilis ATCC 6633	Micrococcus luteus ATCC 9341	B. subtilis ATCC 6633[b]
1	$CH_3(CH_2)_5CO-$	H	H	17	15	21	16
2	$CH_3(CH_2)_6CO-$	H	H	23	18	23	20
3	$CH_3(CH_2)_7CO-$	H	H	20	16	18	27
4	$CH_3(CH_2)_8CO-$	H	H	24	20	22	29
5	$CH_3(CH_2)_9CO-$	H	H	19	19	21	21
6	$CH_3(CH_2)_{10}CO-$	H	H	25	20	19	32
7	$CH_3(CH_2)_{11}CO-$	H	H	21	17	19	29
8	$CH_3(CH_2)_{12}CO-$	H	H	22	21	21	28
9	$CH_3(CH_2)_{13}CO-$	H	H	20	19	19	24
10	$CH_3(CH_2)_{14}CO-$	H	H	19	17	17	23
11	$CH_2=CH-(CH_2)_8CO-$	H	H	21	17	20	25
12	$CH_3(CH_2)_3CH=CH(CH_2)_7CO-$	H	H	22	19	20	30
13	$CH_3CH_2CH=CHCH_2CH=CH-CH_2CH=CH(CH_2)_7CO-$	H	H	15	13	20	14

TABLE 7 contd.

Antibacterial Activity of Formula 1 Compounds by the Agar-Plate Disc-Diffusion Test

Compound No.	R^d	R^1	R^2	Size of Zone of Inhibition (mm)[a] Staphylococcus aureus ATCC 6738P	Bacillus subtilis ATCC 6633	Micrococcus luteus ATCC 9341	B. subtilis ATCC 6633[b]
14	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	CH_3CO-	21	18	20	25
15	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	$HOCO(CH_2)_2CO-$	21	18	19	25
16	$CH_3(CH_2)_6CO-$	H	$CH_3(CH_2)_6CO-$	11	tr[c]	10	17
17	$CH_3(CH_2)_9CO-$	H	$CH_3(CH_2)_9CO-$	17	13	14	20
18	$CH_3(CH_2)_{11}CO-$	H	$CH_3(CH_2)_{11}CO-$	12	tr	-	13
19	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	t-BOC	14	11	10	22
20	Cbz	H	$CH_3(CH_2)_{10}CO-$	15	10	17	23

[a] Compounds were suspended in water at a concentration of 1 mg/ml; a 7-mm disc was dipped into the suspension and then placed on the agar surface; incubation: 24-48 hours at 25-37°C.

[b] Crown on minimal nutrient agar

[c] tr = trace

[d] R^3, R^4, R^5 = H

The results of antibacterial testing of representative compounds of formula I by standard agar-dilution tests are summarized in Table 8. In Table 8 activity is measured by the minimal inhibitory concentration (MIC), i.e. the lowest concentration of compound at which growth of the microorganism is inhibited by the test compound.

Table 8

Antibiotic Activity of A-21978C Cyclic Peptides

Test Organism	MIC Values of Test Compounds[a]										
	1	2[d]	3	4[e]	5[d]	6[e]	7	8	9	10	11
Staphylococcus aureus X1.1	8	4	2	1,0.5	0.25	0.5,0.5	0.125	0.5,1	1	1	0.5
" " V41[b]	8	4	2	1,0.5	0.25	0.5,1	0.125	0.5,2	1	1	0.5
" " X400[c]	8	8	4	2,1	0.5	1,1	0.5	1,2	2	2	0.5
" " S13E	8	4	2	1,0.5	0.5	0.5,0.5	0.25	1,2	1	1	0.25
Staphylococcus epidermidis EPI1	16	8	4	2,1	0.5	1,1	0.5	1,4	2	2	1
" " EPI2	8	4	2	1,0.5	0.5	1,1	0.5	2,2	4	2	1
Streptococcus pyogenes C203	2	1	0.25	0.25, 0.125	0.125	0.125, 0.25	0.25	0.5,2	1	1	0.5
" pneumoniae Park I	8	8	2	1,0.5	0.25	0.25, 0.25	0.25	0.03, 0.125	0.06	0.06	0.25
" Group D X66	128	64	32	16,16	2	2,4	0.03	0.5,2	2	2	1
" " 9960	128	16	8	2,2	0.5	0.5,0.5	32	0.125, >128	0.25	0.25	0.5

[a]MIC in mcg/ml; compound numbers from Tables II, IV and VI and Example 36

[b]Penicillin-resistant strain

[c]Methicillin-resistant-strain

[d]Median value from three tests

[e]Two experiments

Table 8 continued

Test Organism	MIC Values of Test Compounds[a]										
	12	13	14[e]	15[e]	16	17[d]	18	20	21	22[d]	23
Staphylococcus aureus X1.1	1	>128	4,4	4,4	8	1	8	8	1	0.5	8
" " V41[b]	1	>128	4,4	8,8	16	4	64	16	4	1	8
" " X400[c]	2	>128	8,8	4,8	8	4	>128	32	32	1	16
" " S13E	1	>128	8,4	4,8	8	1	>128	8	16	0.5	8
Staphylococcus epidermidis EPI1	2	>128	8,8	8,8	16	8	>128	32	2	1	8
" " EPI2	2	>128	8,4	8,8	16	8	>128	32	2	1	8
Streptococcus pyogenes C203	0.5	>128	1,1	1,1	4	0.25	1	4	0.125	0.5	4
" pneumoniae Park I	0.5	>128	2,2	2,4	4	0.125	0.5	--	0.5	1	8
" Group D X66	2	>128	64,32	64,64	>128	8	>128	128	>128	4	32
" " 9960	1	>128	32,8	32,32	64	8	>128	64	128	1	8

The A-21978C cyclic peptides of formula 1 have shown in vivo antimicrobial activity against experimental bacterial infections. When two doses of test compound were administered subcutaneously or orally to mice in illustrative infections, the activity observed was measured as an ED_{50} value [effective dose in mg/kg to protect fifty percent of the test animals: See Warren Wick, et al., J. Bacteriol. 81, 233-235 (1961)]. The ED_{50} values observed for representative A-21978C compounds of formula 1 are given in Table 9.

Table 9

In Vivo Activity of A-21978C Cyclic Peptides

Compound No.	Formula 1 Compound R^e	R^1	R^2	ED_{50} Values[a] Staphylococcus aureus Subcutaneous	Streptococcus pyogenes Subcutaneous	Streptococcus pyogenes Oral
1	$CH_3(CH_2)_5CO-$	H	H	2.65	1.49,5.1[b]	NT[c]
2	$CH_3(CH_2)_6CO-$	H	H	3.75,1.4	<2.2,0.65,1.9[d]	>200
3	$CH_3(CH_2)_7CO-$	H	H	0.5	0.14,0.243	92,117
4	$CH_3(CH_2)_8CO-$	H	H	0.28	0.03	66
5	$CH_3(CH_2)_9CO-$	H	H	0.37	0.13	138
6	$CH_3(CH_2)_{10}CO-$	H	H	0.44	0.05	69
7	$CH_3(CH_2)_{11}CO-$	H	H	0.54	0.046	45
8	$CH_3(CH_2)_{12}CO-$	H	H	1.17,3.08	0.98,0.20,<0.54	<50,<200
9	$CH_3(CH_2)_{13}CO-$	H	H	8.3	<0.5,0.18	>200
10	$CH_3(CH_2)_{14}CO-$	H	H	>1.1	0.18	>200
11	$CH_2=CH-(CH_2)_8CO-$	H	H	0.93	0.068	138
12	$CH_3(CH_2)_3CH=CH(CH_2)_7CO-$	H	H	3.28	0.134	75
14	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	CH_3CO-	1.3	2.2,1.9	163,>200
15	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	$HOCO-(CH_2)_2CO-$	6.27	1.4	>200
17	$CH_3(CH_2)_9CO-$	H	$CH_3(CH_2)_9-CO-$	>35	2.85	>200
22	$CH_3(CH_2)_{11}-$	H	H	1.25	1.1,0.85	>200

[a] mg/kg x 2, [b] Two Experiments, [c] Not Tested, [d] Three Experiments, [e] R^3, R^4, R^5 = H

The results of toxicity tests on some A-21978C cyclic peptides of formula 1 are summarized in Table 10.

Table 10

Toxicity of A-21978C Cyclic Peptides

Compound No.	Formula 1 Compound R^c	R^1	R^2	LD_{50} (mg/kg) in Mice[a]
1	$CH_3(CH_2)_5CO-$	H	H	>600
2	$CH_3(CH_2)_6CO-$	H	H	>600
3	$CH_3(CH_2)_7CO-$	H	H	>600
4	$CH_3(CH_2)_8CO-$	H	H	>300
5	$CH_3(CH_2)_9CO-$	H	H	>600
6	$CH_3(CH_2)_{10}CO-$	H	H	144,265[b]
7	$CH_3(CH_2)_{11}CO-$	H	H	112.5
8	$CH_3(CH_2)_{12}CO-$	H	H	62.5,<150
9	$CH_3(CH_2)_{13}CO-$	H	H	56.25
10	$CH_3(CH_2)_{14}CO-$	H	H	50
11	$CH_2=CH-(CH_2)_8CO-$	H	H	$\geq$500
12	$CH_3(CH_2)_3CH=CH(CH_2)_7CO-$	H	H	450
14	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	CH_3CO-	>600,600
15	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	H	$HOCO(CH_2)_2CO-$	450,600
16	$CH_3(CH_2)_6CO-$	H	$CH_3(CH_2)_6CO-$	>600
17	$CH_3(CH_2)_9CO-$	H	$CH_3(CH_2)_9CO-$	94
22	$CH_3(CH_2)_{11}-$	H	H	>300

[a]Administered intravenously, [b]Two experiments, [c]R^3, R^4, R^5 = H

Preparations 1-9

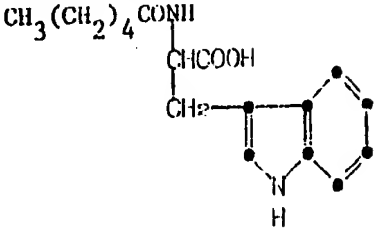
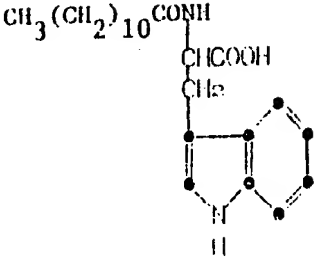
The preparation of a number of useful N-alkanoylamino acids is described in U.S. Patent 4,293,483. Such compounds are prepared according to the following general procedure:

The appropriate alkanoic acid chloride is added dropwise to the appropriate amino acid (1:1 mole ratio) dissolved in pyridine. The amount of pyridine used should be sufficient to make the concentration of reactants between 0.1 to 0.2M. The solution is stirred at room temperature for about 3 to 6 hours, after which it is poured into a large volume of water. The product precipitates from solution and is collected by filtration and crystallized from methanol.

Other N-alkanoylamino acids prepared by this procedure are summarized in Table 11.

TABLE 11

Preparation of N-Alkanoyl Amino Acids

Prep. No.	Alkanoic acid chloride		Amino Acid		N-Alkanoyl Amino Acid	
	Formula	wt. (g)	Formula	wt. (g)	Formula	wt. (g)
1	$CH_3(CH_2)_6COCl$	3.25	L-phenylalanine	3.30	$CH_3(CH_2)_6CONHCH(CH_2C_6H_5)COOH$	4.85
2	$CH_3(CH_2)_7COCl$	2.0	"	1.82	$CH_3(CH_2)_7CONHCH(CH_2C_6H_5)COOH$	2.8
3	$CH_3(CH_2)_8COCl$	3.9	"	3.30	$CH_3(CH_2)_8CONHCH(CH_2C_6H_5)COOH$	5.35
4	$CH_3(CH_2)_9COCl$	4.0	"	3.23	$CH_3(CH_2)_9CONHCH(CH_2C_6H_5)COOH$	4.5
5	$CH_3(CH_2)_{10}COCl$	6.54	"	4.95	$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)COOH$	5.2
6	$CH_3(CH_2)_{11}COCl$	2.0	"	1.42	$CH_3(CH_2)_{11}CONHCH(CH_2C_6H_5)COOH$	1.7
7	$CH_3(CH_2)_{12}COCl$	4.8	"	3.30	$CH_3(CH_2)_{12}CONHCH(CH_2C_6H_5)COOH$	6.6
8	$CH_3(CH_2)_4COCl$	6.2	L-tryptophan	10.2		6.82
9	$CH_3(CH_2)_{10}COCl$	10.9	"	10.2		13.8

Preparations 10-19

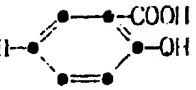
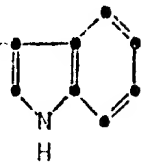
The 2,4,5-trichlorophenyl esters of various N-alkanoylamino acids are described in U.S. Patent 4,293,483. Such compounds are prepared according to the following general procedure:

The N-alkanoylamino acid (1 mole), 2,4,5-trichlorophenol (1.1 mole), and DCC (1 mole) are dissolved in dichloromethane, diethyl ether or THF. The solution is stirred at room temperature for about 16 to about 20 hours after which it is filtered. The filtrate is taken to dryness, and the product is crystallized from either acetonitrile-water or diethyl ether-petroleum ether.

The preparation of other 2,4,5-trichlorophenyl esters of N-alkanoylamino acids prepared by this method is summarized in Table 12.

TABLE 12

Preparation of 2,4,5-Trichlorophenyl Esters

Preparation No.	N-Alkanoyl Amino Acid Formula	wt (g)	2,4,5-Trichlorophenyl Ester Product wt (g)
10	$CH_3(CH_2)_6CONHCH(CH_2C_6H_5)COOH$	2.9	4.1
11	$CH_3(CH_2)_7CONHCH(CH_2C_6H_5)COOH$	2.8	3.86
12	$CH_3(CH_2)_8CONHCH(CH_2C_6H_5)COOH$	3.19	1.5
13	$CH_3(CH_2)_9CONHCH(CH_2C_6H_5)COOH$	3.29	5.01
14	$CH_3(CH_2)_{11}CONHCH(CH_2C_6H_5)COOH$	1.7	2.01
15	$CH_3(CH_2)_{12}CONHCH(CH_2C_6H_5)COOH$	3.75	4.18
16	$CH_3(CH_2)_{10}CONH$– 	2.1	1.6
17	$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)COOH$	3.47	3.98
18	$CH_3(CH_2)_4CONHCH(COOH)-CH_2$– 	6.04	1.87
19	$CH_3(CH_2)_{10}CONHCH(COOH)-CH_2$– 	7.76	4.03

EXAMPLES 38-70

The N-alkanoylamino acid derivatives of A-21978C of formula 1 are prepared according to the following general procedure which involves acylating the nucleus using the activated-ester method:

A solution of N_{Orn}-t-BOC-blocked-A-21978C nucleus (t-BOC nucleus) in DMF was treated with the 2,4,5-trichlorophenyl ester ("active ester") of the corresponding N-alkanoylamino acid. The reaction mixture was stirred at room temperature for about 18 to about 24 hours under an atmosphere of nitrogen and then was evaporated to dryness under reduced pressure to give a residue. A small amount of methanol was added to the residue; a solid (N,N'-dicyclohexylurea) which did not dissolve in the methanol was removed by filtration and discarded. The filtrate was evaporated under vacuum to give a solid, the crude N_{Orn}-t-BOC-N_{Trp}-acyl-A-21978C analog. This analog was purified using a "Prep LC/System 500" unit (Waters Associates, Inc., Milford, MA) equipped with a Prep Pak-500/C_{18} column (Waters Associates Inc.) as a stationary phase. The column was eluted isocratically, using a water:methanol:acetonitrile (2:1:2) solvent system containing 0.1% pyridinium acetate, and eluting one 250-ml fraction per minute for approximately 40 fractions. Amounts of sample applied varied from about 1 g to about 5 g. Early fractions containing unreacted starting materials were discarded. The desired product was always the major peak (using a UV detector) following the early

fractions. Individual fractions were pooled on the basis of TLC [reversed-phase silica gel (C_{18}), a water:-methanol:acetonitrile (3:3:4) solvent system, and Von Urk spray for detection] and bioautographic analysis [silica-gel TLC plates (Merck), an acetonitrile: acetone:water (2:2:1) solvent system and Micrococcus luteus as the assay organism].

The N_{Orn}-t-BOC-N_{Trp}-acyl-A-21978C analog, obtained as a single component by this method, was lyophilized and treated with anhydrous trifluoroacetic-acid (10 ml per 0.3-0.5 g of analog) containing 2% anisole at 0°C. After about five minutes the reaction mixture was evaporated to dryness under reduced pressure. The residue obtained was triturated with a small amount of ether. The solid precipitate was collected and air-dried. This material was dissolved in water; the pH of the solution was raised to about 6 to 7 by the addition of pyridine; and the solution was then lyophilized. The resulting product was obtained as a single component and was characterized by its chromatographic properties and its amino-acid analysis.

Table 13 summarizes a group of N_{Trp}-alkanoyl-aminoacyl A-21978C derivatives prepared by this procedure.

TABLE 13

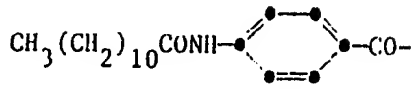
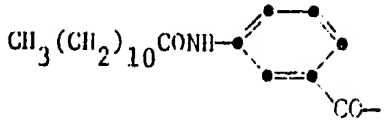
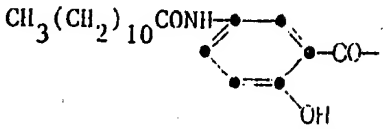
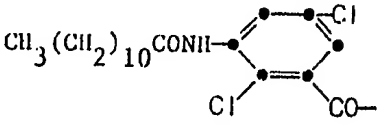
Example No.	Product[a] R in formula 1	Ester (mg)	A-21978C Nucleus (mg)	t-BOC A-21978C Product (mg)[b]	Product (mg)	R_f[c]
38	(D)$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)CO-$	900	900[d]	556	436	0.82
39	$CH_3(CH_2)_{10}CONH(CH_2)_4-CO-$	900	900[d]	489	485	0.65
40	$CH_3(CH_2)_{10}CONH(CH_2)_{10}-CO-$	600	900[d]	326	242	0.83
41		416	1000[e]	--	195	0.34
42		900	900[d]	352	263	0.57
43		800	800[d]	76	24	0.23
44		800	800[d]	389	312	0.17
45	(L)$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)CO-$	800	800[d]	421	324	0.82

TABLE 13 cont.

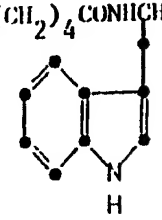
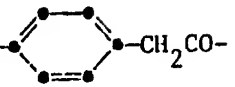
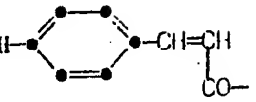
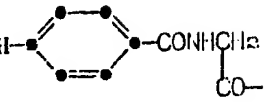
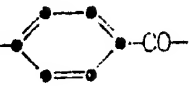
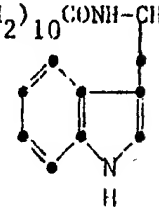
Example No.	Product[a] R in formula 1	Ester (mg)	A-21978C Nucleus (mg)	t-BOC A-21978C Product (mg)[b]	Product (mg)	R_f[c]
46	(L)$CH_3(CH_2)_4CONHCHCO-$ 	1000	1000	--	230	0.68
47	$CH_3(CH_2)_{10}CONH-$  $-CH_2CO-$	900	900[d]	633	485	0.74
48	trans-$CH_3(CH_2)_{10}CONH-$  $-CH=CH-CO-$	800	800[d]	245	186	0.58
49	$CH_3(CH_2)_{10}CONH-$  $-CONHCH_2-CO-$	350	700[d]	376	304	0.38
50	$CH_3(CH_2)_{10}CONH-$ 	900	900[d]	320	218	0.65
51	$CH_3(CH_2)_5CONH(CH_2)_{10}-CO-$	900	900[d]	503	428	0.60
52	$CH_3(CH_2)_{12}CONH-$  $-CO-$	900	900[d]	273	212	0.83

TABLE 13 cont.

Example No.	Product[a] R in formula 1	Ester (mg)	A-21978C Nucleus(mg)	t-BOC A-21978C Product(mg)[b]	Product(mg)	R_f[c]
53	(L)-$CH_3(CH_2)_{10}CONH-CH-CO-$ 	1000	1000[d]	426	286	0.73
54	(L)$CH_3(CH_2)_6CONHCH(CH_2C_6H_5)CO-$	800	800[d]	444	343	0.48
55	(L)$CH_3(CH_2)_7CONHCH(CH_2C_6H_5)CO-$	800	800[d]	412	312	0.50
56	(L)$CH_3(CH_2)_8CONHCH(CH_2C_6H_5)CO-$	800	800[d]	519	453	0.52
57	(L)$CH_3(CH_2)_9CONHCH(CH_2C_6H_5)CO-$	800	800[d]	371	287	0.53
58	(L)$CH_3(CH_2)_{11}CONHCH(CH_2C_6H_5)CO-$	800	800[d]	412	335	
59	(L)$CH_3(CH_2)_{12}CONHCH(CH_2C_6H_5)CO-$	800	800[d]	468	328	

[a] R^{1-5}=H, [b] N_{Orn}-t-BOC-N_{Trp}-Acyl-Nucleus,

[c] Thin-layer chromatography on silica gel (Merck), using a water:CH_3CN:acetone (1:2:2) solvent system

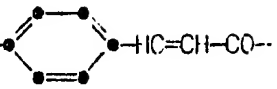
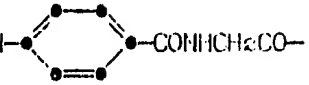
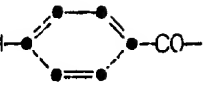
[d] N_{Orn}-t-BOC-Nucleus,

[e] A21978C Nucleus.

Other N-alkanoylamino acid derivatives of formula I prepared in a similar manner are summarized in Table 14.

TABLE 14

A-21978C Cyclic Peptides of Formula 1

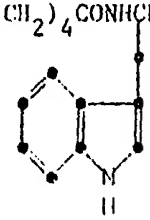
Example No.	R	R^{2a}
60	cis-$CH_3(CH_2)_{10}CONH$-  -HC=CH-CO-	H
61	$CH_3(CH_2)_{10}CONH$-  -$CONHCH_2CO$-	H
62	H	$CH_3(CH_2)_{10}CONH$-  -CO-
63	H	$CH_3(CH_2)_9CONHCH(CH_2C_6H_5)CO$-

[a] R^1, R^3, R^4, R^5 = H

Table 15 summarizes a group of A-21978C derivatives prepared according to the general procedure, but using A-21978C factors as starting materials.

TABLE 15

Diacyl Derivatives of A-21978C

Example No.	R^b in formula 1	R^2 in formula 1	Starting A-21978C Factor	Ester (mg)	Factor (mg)	Product (mg)	R_f [a]
64	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	(L)-$CH_3(CH_2)_4CONHCH(CO-)$ 	C_1	100	48	43	0.81
65	$CH_3CH(CH_3)(CH_2)_8CO-$	"	C_2	100	48	25	0.73
66	$CH_3CH_2CH(CH_3)(CH_2)_8CO-$	"	C_3	400	1000	413	0.87
67	$CH_3CH_2CH(CH_3)(CH_2)_6CO-$	$H_2N(CH_2)_{10}-CO-$	C_1	1000	1000	732	0.65

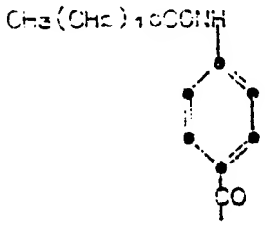
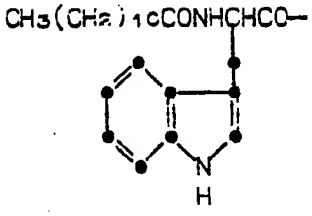
[a] TLC on silica gel (Merck) using a water:CH_3CN:acetone (1:2:2)solvent system

[b] R^1, R^3, R^4, R^5 = H

Other N_{Trp}-N_{Orn}-diacyl derivatives of A-21978C prepared according to the general procedure are listed in Table 16.

TABLE 16

Diacyl Derivatives of A-21978C

Example No.	R in Formula 1[a]	R^2 in Formula 1
68		
69		t-BOC
70	$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)CO-$	t-BOC

[a] R^1, R^3, R^4, R^5 = H

EXAMPLE 71

Preparation of N_{Trp}-[N-(n-Decanoyl)-L-phenylalanyl]-A-21978C Nucleus (Compound of Example 56)

This example illustrates the large-scale preparation of compounds by the active-ester method.

A. Preparation of N-(n-Decanoyl)-L-Phenylalanyl-2,4,5-Trichlorophenolate

A solution of N-(n-decanoyl)-L-phenylalanine (31.9 g, 0.1 mole) and 2,4,5-trichlorophenol (19.7 g, 0.1 mole) in 1 liter of anhydrous ether was treated with N,N'-dicyclohexylcarbodiimide (20.6 g, 0.1 mole). The reaction was stirred overnight at room temperature. The precipitated N,N'-dicyclohexylurea was removed by filtration and discarded. The filtrate was concentrated under vacuum to dryness. The residue obtained was triturated with ether, and the solids (residual cyclohexylurea) were removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The residue was crystallized from acetonitrile to give 36.9 g of crystalline N-(n-decanoyl)-L-phenylalanyl 2,4,5-trichlorophenolate, m.p. 122-124°C.

B. Preparation of N_{Trp}-[N-(n-Decanoyl)-L-phenylalanyl]-N_{Orn}-t-BOC-A-21978C Nucleus

A solution of N-(n-decanoyl)-L-phenylalanyl 2,4,5-trichlorophenolate (10 g, 0.02 mole), N_{Orn}-t-BOC-A-21978C nucleus (10 g, 0.006 mole) in anhydrous DMF (1 L) was stirred at room temperature for 96 hours under an atmosphere of nitrogen. The solvent was removed by evaporation under reduced pressure. The residual material was stirred with a mixture of diethyl ether (800 ml) and chloroform (200 ml) for 2 hours. The product was separated by filtration to give a light brown powder (10.3 g). This material (9.9 g) was

dissolved in methanol (200 ml) and purified by preparative HPLC, using a "Prep LC/System 500" unit and a PrepPak-500/C_{18} Column as the stationary phase. The column was eluted isocratically, using a water:methanol:acetonitrile (2:1:2) solvent system and collecting 250-ml fractions at a rate of one fraction per minute. The desired compound was eluted in the 9th through the 22nd fractions.

Fractions were combined on the basis of TLC [reversed phase silica gel/C_{18}; developed with water:methanol:acetonitrile (3:3:4); detected with Van Urk spray]. Combined fractions were examined by bioautography [silica gel TLC acetonitrile:acetone:water (2:2:1) solvent system and Micrococcus luteus as the detecting organism] and were shown to consist of a single bioactive component. This procedure gave 6.02 g of N_{Trp}-[N-(n-decanoyl)-L-phenylalanyl]-N_{Orn}-t-BOC-A-21978C nucleus [compound of formula 1: R = N-(n-decanoyl-L-phenylalanyl); R^2= t-BOC].

C. Preparation of N_{Trp}-[N-(n-Decanoyl)-L-phenylalanyl]-A-21978C Nucleus

A flask (100 ml) was cooled to 5°C. in an icebath. N_{Trp}-[N-(n-decanoyl)-L-phenylalanyl]-N_{Orn}-t-BOC-A-21978C nucleus (6.02 g, 0.008 mole), prepared as described in Section B, and then anhydrous trifluoroacetic acid containing 2% anisole (50 ml) were added to the flask. The mixture, which went into solution in approximately two minutes, was stirred under an atmosphere of nitrogen for ten minutes. The solution was

evaporated to dryness under reduced pressure at below 40°C. to give a gummy solid which was triturated twice with a diethyl ether:dichloromethane (4:1) solution (two 100-ml volumes). The solids were collected by filtration and washed with diethyl ether to give the TFA salt. This was dissolved in water (50 ml), and the pH of the solution was adjusted to 5.4 with pyridine. The solution was then lyophilized to give 6.1 g of off-white lyophilizate.

The lyophilizate, dissolved in methanol (35 ml), was purified using a reverse-phase C_{18} silica-gel column (Waters Associates, Prep 500), eluting in stepwise gradient with H_2O:CH_3OH:CH_3CN containing 0.1% pyridinium acetate at ratios of 3:1:2, 2:1:2 and 1:2:2 and collecting fractions having a volume of 250 ml. The desired product was eluted during the 2:1:2 elution. The fractions containing the product were lyophilized to give 2.23 g of cream-colored N_{Trp}-[N-(n-decanoyl)-L-phenylalanyl]-A-21978C nucleus (compound of formula 1: R = N-(n-decanoyl)-L-phenylalanyl; R^{1-5} = H).

The product was evaluated by analytical HPLC [reversed-phase C_{18} silica-gel column, MeOH:CH_3CN:H_2O:PyOAc (15:35:49:1) solvent and UV detection at 230 nm], by TLC [reversed-phase C_{18} silica-gel plates (Whatman), H_2O:CH_3OH:CH_3CN (3:3:4) solvent and Van Urk spray and short-wave UV for detection] and by bioautography [silica-gel TLC (Merck), an H_2O:CH_3CN:acetone (1:2:2) solvent, and Micrococcus luteus as the detecting organism]. Each of these methods demonstrated that the product was homogenous. Substitution at the tryptophan

N-terminus position was confirmed by 360 MHz PMR. Amino-acid analysis confirmed the incorporation of one equivalent of L-phenylalanine into the product.

EXAMPLE 72

The antibiotic activity of the compounds of formula I can be demonstrated in vitro, using standard agar-dilution tests. The results of the antibacterial testing of representative compounds of formula I are set forth in Table 17. In Table 17 activity is measured by the minimal inhibitory concentration (MIC), i.e. the lowest concentration of compound at which growth of the microorganism is inhibited by the test compound.

Table 17

Antibiotic Activity of A-21978C Cyclic Peptides

Test Organism	MIC[a] of Test Compound[b]			
	38	39[e]	40	41[f]
Staphylococcus aureus X1.1	0.5	4	2	4,8
" " V41[c]	0.5	4	2	4,16
" " X400[d]	1	4	8	8,16
" " S13E	0.5	4	2	4,8
Staphylococcus epidermidis EPI1	2	4	8	16,64
" " EPI2	1	2	8	16,64
Streptococcus pyogenes C203	0.125	1	2	4,8
" pneumoniae Park I	0.125	4	0.5	4,16
" Group D X66	1	32	8	32, >64
" " 9960	0.5	8	8	8,32

[a] MIC in mcg/ml

[b] Numbers = example numbers in Tables 13-16

[c] Penicillin-resistant strain

[d] Methicillin-resistant-strain

[e] Median of five experiments

[f] Two experiments

[g] Median of three experiments

Table 17 cont'd.

Antibiotic Activity of A-21978C Cyclic Peptides

Test Organism			MIC[a] of Test Compound[b]									
			42	43	44	45	46[f]	47[f]	48	49	50	51
Staphylococcus	aureus	X1.1	0.5	1	0.5	0.25	32,64	0.5,0.25	0.5	1	0.25	4
"	"	V41[c]	0.5	2	1	0.25	64,128	0.5,0.5	0.25	2	0.25	4
"	"	X400[d]	1	4	2	0.5	64,128	1,0.5	2	4	0.5	8
"	"	S13E	0.5	2	0.5	0.25	32,64	0.5,0.5	0.5	2	0.25	4
Staphylococcus	epidermidis EPI1		2	2	2	1	128, >128	2,1	0.5	4	0.5	8
"	"	EPI2	1	2	2	1	128, >128	1,0.5	0.5	4	0.25	8
Streptococcus	pyogenes	C203	0.25	0.5	1	0.25	16,16	0.125, 0.06	0.125	2	0.03	2
"	pneumoniae	Park 1	0.25	2	0.125	0.125	32,64	0.25, 0.25	0.06	1	0.03	8
"	Group D	X66	4	16	2	1	>128, >128	4,4	2	16	1	>128
"	"	9960	1	2	2	0.25	128, >128	2,1	4	4	0.25	64

Table 17 cont'd.

Antibiotic Activity of A-21978C Cyclic Peptides

Test Organism	MIC[a] of Test Compound[b]									
	52	53	54	55	56[g]	57	58	59	60	61
Staphylococcus aureus X1.1	1	0.5	4	2	0.5	0.5	0.5	0.5	0.5	1
" V41[c]	2	0.5	8	2	0.5	0.5	0.5	1	1	2
" X400[d]	8	1	8	4	1	2	2	2	4	4
" S13E	2	0.5	8	2	0.5	0.5	0.5	1	1	2
Staphylococcus epidermidis EPI1	4	1	8	4	1	2	2	4	2	4
" EPI2	4	1	8	2	0.5	2	2	4	2	4
Streptococcus pyogenes C203	1	0.5	2	0.5	0.25	0.125	0.125	0.25	0.25	2
" pneumoniae Park I	0.25	0.125	-	-	0.75	-	-	-	0.25	1
" Group D X66	4	1	128	32	8	4	1	1	4	16
" 9960	4	0.5	32	8	2	2	0.25	0.5	2	4

Table 17 cont'd.

Antibiotic Activity of A-21978C Cyclic Peptides

Test Organism		MIC[a] of Test Compound[b]								
		62	63	64	65	66[f]	67	68[f]	69	70
Staphylococcus aureus	X1.1	8	8	0.5	0.5	0.5,1	1	16,2	1	0.5
" "	V41[c]	16	>128	2	2	4,4	1	32,4	1	2
" "	X400[c]	16	>128	1	1	1,1	2	32,8	2	2
" "	S13E	8	16	0.5	0.5	0.5,1	1	16,4	1	1
Staphylococcus epidermidis EPI1		16	>128	4	4	8,16	2	128,16	4	8
" "	EPI2	16	>128	4	4	8,16	1	64,8	4	8
Streptococcus pyogenes	C203	4	1	0.5	0.5	1,0.5	0.25	8,4	1	1
" pneumoniae	Park I	8	-	0.5	0.25	0.25,0.5	0.25	8,4	0.5	0.5
" Group D	X66	128	>128	8	4	8,16	64	>128, 32	8	16
" "	9960	32	32	4	4	32,32	32	64,8	4	4

The A-21978C cyclic peptides of formula 1 have shown in vivo antimicrobial activity against experimental bacterial infections. When two doses of test compound were administered subcutaneously or orally to mice in illustrative infections, the activity observed was measured as an ED_{50} value [effective dose in mg/kg to protect fifty percent of the test animals: See Warren Wick, et al., J. Bacteriol. 81, 233-235 (1961)]. The ED_{50} values observed for A-21978C compounds are given in Tables 18 and 19.

TABLE 18

In Vivo Activity of A-21978C Cyclic Peptides

Example No.	Formula 1 Compound[a] R	ED_{50} Values[b] Staphylococcus aureus Subcutaneous	Streptococcus pyogenes Subcutaneous	Streptococcus pyogenes Oral
38	(D)$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)CO-$	1.4, 2.05[c]	<0.25, 0.21	>200
39	$CH_3(CH_2)_{10}CONH(CH_2)_4-CO-$	0.65, 0.93	<0.25, 0.107	117
40	$CH_3(CH_2)_{10}CONH(CH_2)_{10}-CO-$	>18	6.2	>200
41	$CH_3(CH_2)_{10}CONH$-(1,4-C$_6$H$_4$)-$CO-$	<5	18.8	>200
42	$CH_3(CH_2)_{10}CONH$-(1,2-C$_6$H$_4$)-$CO-$	1.67	>0.25, 0.46	>200
44	$CH_3(CH_2)_{10}CONH$-(2,5-dichloro-1,4-C$_6$H$_3$)-$CO-$	19	0.23	>200

TABLE 18 cont'd.

In Vivo Activity of A-21978C Cyclic Peptides

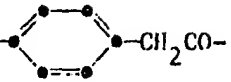
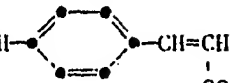
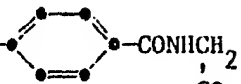
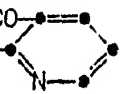
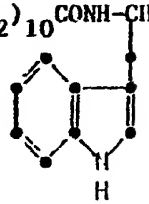
Example No.	Formula 1 Compound[a] R	ED_{50} Values[b] Staphylococcus aureus Subcutaneous	Streptococcus pyogenes Subcutaneous	Streptococcus pyogenes Oral
45	(L)$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)CO-$	2.35	0.32	150
47	$CH_3(CH_2)_{10}CONH$–  –CH_2CO-	3.56, 4.12[c]	0.69	>200
48	$CH_3(CH_2)_{10}CONH$–  –$CH=CH-CO-$	0.65	0.04	59
49	$CH_3(CH_2)_{10}CONH$–  –$CONHCH_2CO-$	2.3	<0.25, 0.12[c]	>200
50	$CH_3(CH_2)_{10}CONH$– 	0.54	0.05	69
51	$CH_3(CH_2)_5CONH(CH_2)_{10}-CO-$	>18	>9	>200

TABLE 18 cont'd.

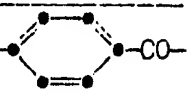
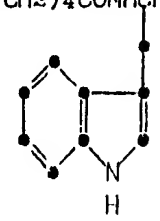
In Vivo Activity of A-21978C Cyclic Peptides

Example No.	Formula 1 Compound[a] R	ED_{50} Values[b] Staphylococcus aureus Subcutaneous	Streptococcus pyogenes Subcutaneous	Streptococcus pyogenes Oral
52	$CH_3(CH_2)_{12}CONH$–  –CO–	>18	1.02	>200
53	(L)-$CH_3(CH_2)_{10}CONH$–CH–CO– 	5.19	3.14	200
54	(L)$CH_3(CH_2)_6CONHCH(CH_2C_6H_5)CO$–	2.58	1.48	>200
55	(L)$CH_3(CH_2)_7CONHCH(CH_2C_6H_5)CO$–	1.38	0.59	184
56	(L)$CH_3(CH_2)_8CONHCH(CH_2C_6H_5)CO$–	0.7, 0.98[c]	0.39	>200
57	(L)$CH_3(CH_2)_9CONHCH(CH_2C_6H_5)CO$–	1.25	0.35	>200
58	(L)$CH_3(CH_2)_{11}CONHCH(CH_2C_6H_5)CO$–	0.76	0.14	>200
59	(L)$CH_3(CH_2)_{12}CONHCH(CH_2C_6H_5)CO$–	4.8	<0.27, 0.36[c]	>200
61	$CH_3(CH_2)_{10}CONH$–  –$CONHCH_2CO$–	2.3	<0.25, 0.12[c]	>200

[a] R^{1-5} = H, [b] mg/kg x 2, [c] Two experiments

TABLE 19

In Vivo Activity of A-21978C Cyclic Peptides

Example No.	Formula 1 Compound R^c	Formula 1 Compound R^2	ED_{50} Values[a] Staphylococcus aureus Subcutaneous	Streptococcus pyogenes Subcutaneous	Streptococcus pyogenes Oral
62	H	$CH_3(CH_2)_{10}CONH$–  –CO–	>70	>22	>200
63	H	$CH_3(CH_2)_8CONHCH(CH_2C_6H_5)CO$–	9.2	>18	>200
66	$CH_3CH_2CH(CH_3)(CH_2)_8CO$–	(L)–$CH_3(CH_2)_4CONHCH$–  –CO–	>2.2, >70[b]	10.6, 6.0	>200

[a] mg/kg x 2

[b] Two experiments

[c] R^1, R^3, R^4, R^5 = H

The results of toxicity tests on some of the A-21978C cyclic peptides are summarized in Table 20.

TABLE 20

Toxicity of A-21978C Cyclic Peptides

Formula 1 Compound

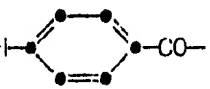
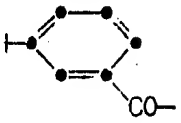
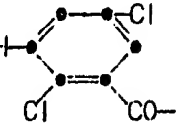
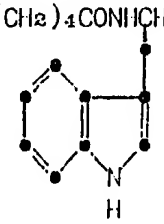
Example No.	R^C	R^2	LD_{50} (mg/kg) in Mice[a]
38	(D)$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)CO-$	H	250
39	$CH_3(CH_2)_{10}CONH(CH_2)_4-CO-$	H	>600
40	$CH_3(CH_2)_{10}CONH(CH_2)_{10}-CO-$	H	600
41	$CH_3(CH_2)_{10}CONH$-  -$CO-$	H	>300
42	$CH_3(CH_2)_{10}CONH$- 	H	277
44	$CH_3(CH_2)_{10}CONH$- 	H	200
45	(L)$CH_3(CH_2)_{10}CONHCH(CH_2C_6H_5)CO-$	H	250
46	(L)$CH_3(CH_2)_4CONHCHCO-$ 	H	450[b]

TABLE 20 cont'd.

Toxicity of A-21978C Cyclic Peptides

Formula 1 Compound

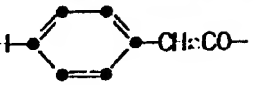
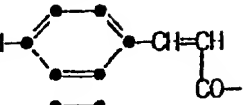
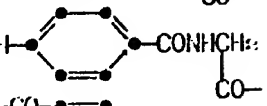
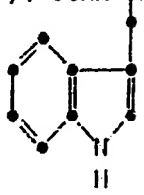
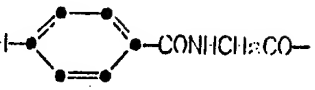
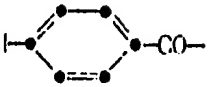
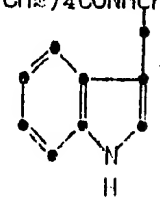
Example No.	R	R^2	LD_{50} (mg/kg) in Mice[a]
47	$CH_3(CH_2)_{10}CONH$–  –CH_2CO–	H	450
48	$CH_3(CH_2)_{10}CONH$–  –$CH{=}CH$–CO–	H	450
49	$CH_3(CH_2)_{10}CONH$–  –$CONHCH_2$–CO–	H	450
50	$CH_3(CH_2)_{10}CONH$– 	H	300
51	$CH_3(CH_2)_8CONH(CH_2)_{10}CO$–	H	>600
52	$CH_3(CH_2)_{12}CONH$–  –CO–	H	250
53	(L)-$CH_3(CH_2)_{10}CONH$–CH–CO– 	H	225
54	(L)$CH_3(CH_2)_8CONHCH(CH_2C_6H_5)CO$–	H	450

TABLE 20 cont'd.

Toxicity of A-21978C Cyclic Peptides

Formula 1 Compound

Example No.	R	R^2	LD_{50} (mg/kg) in Mice[a]
55	(L)$CH_3(CH_2)_7CONHCH(CH_2C_6H_5)CO-$	H	>600
56	(L)$CH_3(CH_2)_8CONHCH(CH_2C_6H_5)CO-$	H	600
57	(L)$CH_3(CH_2)_9CONHCH(CH_2C_6H_5)CO-$	H	400
58	(L)$CH_3(CH_2)_{11}CONHCH(CH_2C_6H_5)CO-$	H	225
59	(L)$CH_3(CH_2)_{12}CONHCH(CH_2C_6H_5)CO-$	H	225
61	$CH_3(CH_2)_{10}CONH$–  –$CONHCH_2CO-$	H	450
62	H	$CH_3(CH_2)_{10}CONH$–  –$CO-$	300
63	H	$CH_3(CH_2)_9CONHCH(CH_2C_6H_5)CO-$	225
66	$CH_3CH_2CH(CH_3)(CH_2)_8CO-$	(L)–$CH_3(CH_2)_4CONHCH-CO-$ 	37.5

[a] Administered intravenously

[b] Material was in suspension

[c] R^1, R^3, R^4, R^5 = H

EXAMPLE 73

A. Preparation of N_{Trp}-p-(n-Dodecyloxy)benzoyl-N_{Orn}-t-BOC-A-21978C Nucleus

A solution of 2,4,5-trichlorophenyl p-(n-dodecyloxy)benzoate (0.9 g), and, A-21978C t-BOC nucleus (0.9 g) in 400 ml of anhydrous dimethylformamide was allowed to stir at room temperature for 120 hours under an atmosphere of nitrogen. The solvent was removed by evaporation under reduced pressure. The residual material was stirred with a mixture of diethyl ether (400 ml) and chloroform (400 ml) for 2 hours. The product was separated by filtration and dried to give a light brown powder (0.962 g). A portion of this material (0.78 g) was dissolved in methanol (200 ml) and purified by preparative HPLC, using a "Prep LC/System 500" unit (Waters Associates, Inc., Milford Mass.) and a Prep Pak-500/C_{18} Column (Waters Associates) as a stationary phase. The column was operated isocratically, using a water:methanol:acetonitrile (2:1:2) solvent system and collecting 250-ml fractions (1 fraction/min.). The desired compound was eluted in the 2nd to the 6th fractions.

Fractions were combined on the basis of TLC [reverse phase/C_{18} silica gel, developed with water:methanol:acetonitrile (3:3:4), detected with Van Urk spray]. Bioautography of the combined fractions, using silica gel TLC, an acetonitrile:acetone:water (2:2:1) solvent, and Staphylococcus aureus as the detecting organism, indicated that the product was a single bioactive component. This procedure gave 0.421 g of N_{Trp}-p-(n-dodecyloxy)benzoyl-N_{Orn}-t-BOC-A-21978C nucleus.

B. Preparation of N_{Trp}-p-(n-dodecyloxy)benzoyl-A-21978C Nucleus

N_{Trp}-p-(n-Dodecyloxy)benzoyl-N_{Orn}-t-BOC-A-21978C nucleus (230 mg) was dissolved in 5 ml of trifluoroacetic acid containing 2% anisole and stirred for 5 minutes at 0°C. The solution was concentrated to an oil under vacuum, and the oil was triturated with Et_2O (100 ml). The solids were separated, air-dried, and taken up in water (10 ml). The pH of this solution was adjusted from 3.25 to 7 by the addition of pyridine. The resulting solution was lyophilized to give 179 mg of white amorphous N_{Trp}-p-(n-dodecyloxy)benzoyl-A-21978C nucleus. This compound has an R_f value of about 0.78 on silica-gel TLC, using an acetonitrile:acetone:water (2:2:1) solvent system and Van Urk spray for detection.

EXAMPLE 74

The antibacterial activity of the compounds of Example 73 can be demonstrated in vitro. The results of the antibacterial testing of these compounds using standard agar-plate disc-diffusion tests are set forth in Table 21. In Table 21 activity is measured by the size (diameter in mm) of the observed zone in which growth of the microorganism is inhibited by the test compound.

TABLE 21

Antibacterial Activity of Formula 1 Compounds by the Agar-Plate Disc-Diffusion Test

Compound		Size of Zone of Inhibition (mm)[a]			
R^c	R^2	Staphylococcus aureus ATCC 6738P	Bacillus subtilis ATCC 6633	Micrococcus luteus ATCC 9341	B. subtilis ATCC 6633[b]
p-(n-dodecyloxy)benzoyl	H	20	13	18	20
p-(n-dodecyloxy)benzoyl	t-BOC	20	10	15	22

[a] Compounds were suspended in water at a concentration of 1 mg/ml; a 7-mm disc was dipped into the suspension and then placed on the agar surface; incubation--24-48 hours at 25-37°C.

[b] Grown on minimal nutrient agar

[c] R^1, R^3, R^4, R^5 = H

The results of the antibacterial testing of a representative benzoic acid derivative, using the standard agar-dilution test, are summarized in Table 22. In Table 22 activity is measured by the minimal inhibitory concentration (MIC), i.e. the lowest concentration of compound which inhibits the growth of the microorganism.

Table 22

Antibiotic Activity of N_{Trp}-p-(n-Dodecyloxy)benzoyl-A-21978C

Test Organism	MIC Values[a]
Staphylococcus aureus X1.1	1
" " V41[b]	1
" " X400[c]	2
" " S13E	1
Staphylococcus epidermidis EPI1	4
" " EPI2	2
Streptococcus pyogenes C203	0.25
" pneumoniae Park I	0.015
" Group D X66	2
" " 9960	1

[a]MIC in mcg/ml

[b]Penicillin-resistant strain

[c]Methicillin-resistant-strain

The representative benzoic acid derivatives of A-21978C nucleus have shown in vivo antimicrobial activity against experimental bacterial infections. When two doses of test compound were administered to mice in illustrative infections, the activity observed was measured as an ED_{50} value [effective dose in mg/kg to protect fifty percent of the test animals: See Warren Wick, et al., J. Bacteriol. 81, 233-235 (1961)]. The ED_{50} values observed are given in Table 23.

Table 23

In Vivo Activity of N_{Trp}-p-(n-Dodecyloxy)benzoyl-A-21978C

Formula I Compound[a]

R	ED_{50} Values[b]			
	Staphylococcus aureus		Streptococcus pyogenes	
N_{Trp}-(p-(n-Dodecyloxy)benzoyl	Subcutaneous		Subcutaneous	Oral
	3.35		0.31	>200

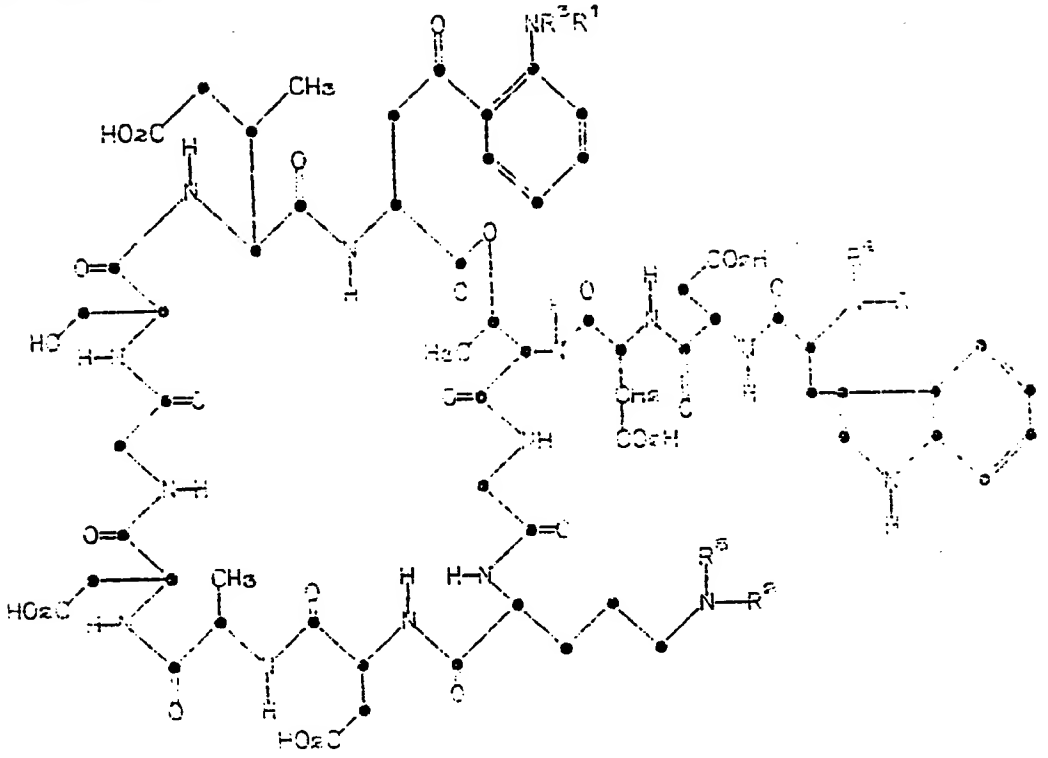
[a] R^{1-5} = H

[b] mg/kg x 2

The acute toxicity of N_{Trp}-p-(n-dodecyloxy)-benzoyl-A-21978C, when administered intravenously to mice and expressed as LD_{50}, was 67.5 mg/kg.

CLAIMS

1. An A-21978C cyclic peptide derivative of formula (1):



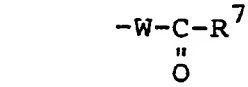
1

in which R, R^1, and R^2 are, independently, hydrogen, 8-methyldecanoyl, 10-methyldodecanoyl, 10-methyl-undecanoyl, the specific C_{10}-alkanoyl group of A-21978C_0 the specific C_{12}-alkanoyl groups of A-21978C factors C_4 or C_5, an amino-protecting group,

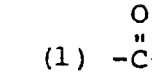
(A) an aminoacyl group of the formula

$$-\overset{\overset{O}{\|}}{C}-Q-NH_2$$

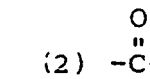
in which Q is C_1-C_{16} alkylene, or an N-alkanoyl-aminoacyl group of the formula



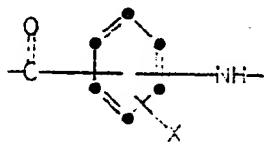
in which W is a divalent aminoacyl radical of the formula:

(1) 

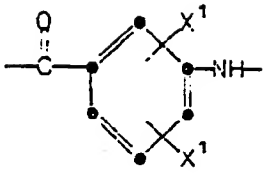
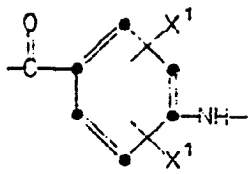
in which A is C_1-C_{10} alkylene or C_5-C_6 cycloalkylene;

(2) 

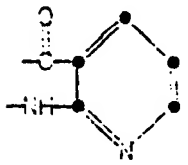
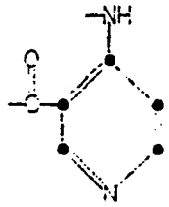
in which R^8 is hydroxymethyl, hydroxyethyl, mercaptomethyl, mercaptoethyl, methylthioethyl, 2-thienyl, 3-indole-methyl, phenyl, benzyl, or substituted phenyl or substituted benzyl in which the benzene ring thereof is substituted with chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3-alkoxy, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

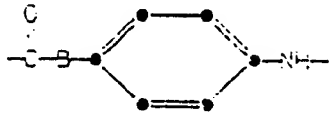
(3) 

in which X is hydrogen, chloro, bromo, iodo, amino, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy, mercapto, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

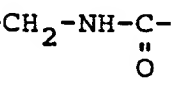
(4)  or 

in which X^1 is chloro, bromo, iodo, amino, hydroxy, C_1-C_3 alkyl or C_1-C_3 alkoxy;

(5)  or  ; or

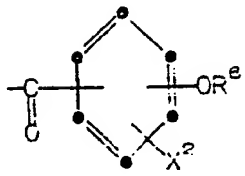
(6) 

in which B is a divalent radical of the formula: $-(CH_2)_n-$ and n is an integer from 1 to 3; $-CH=CH-$; $-CH=CH-CH_2-$; or

 ;

R^7 is C_1-C_{17} alkyl or C_2-C_{17} alkenyl;

(B) a substituted benzoyl group of the formula



in which R^6 is C_8-C_{15} alkyl;
X^2 is hydrogen, chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy or C_1-C_3 alkylthio;

(C) optionally substituted C_2-C_{19} alkanoyl, C_5-C_{19} alkenoyl, C_4-C_{14} alkyl provided that R, R^1 and R^2 groups together must contain at least four carbon atoms;

and in which R^3, R^4 and R^5 (i) may represent hydrogen or (ii) taken together with an appropriate adjacent R, R^1, R^2 group may represent a C_4-C_{14} alkylidene group; provided that when R^1 and R^2 are both selected from hydrogen, R cannot be 8-methyldecanoyl, 10-methyl-undecanoyl, 10-methyldodecanoyl, the specific C_{10}-alkanoyl group of A-21978C factor C_0 or the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5; or a pharmaceutically-acceptable salt thereof.

2. An A-21978C cyclic peptide derivative of formula (1) as claimed in claim 1 in which R is hydrogen, 8-methyldecanoyl, 10-methyldodecanoyl, 10-methylundecanoyl, the specific C_{10}-alkanoyl group of A-21978C_0 or the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_5, an amino-protecting group, an aminoacyl group of the formula

$$-\overset{O}{\overset{\|}{C}}-Q-NH_2$$

wherein Q is C_1-C_{16} alkylene, or an N-alkanoylamino acyl group of the formula $-W-\overset{O}{\overset{\|}{C}}-R^7$ in which:

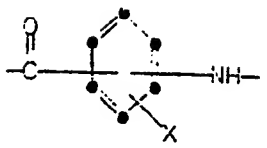
W is a divalent aminoacyl radical of the formula:

(a) $-\overset{O}{\overset{\|}{C}}-A-NH-$

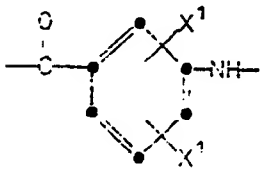
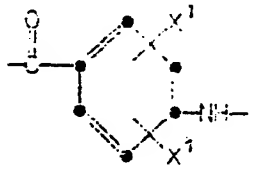
in which A is C_1-C_{10} alkylene or C_5-C_6 cycloalkylene;

(b) $-\overset{O}{\overset{\|}{C}}-\overset{R^8}{\overset{|}{C}}H-NH-$

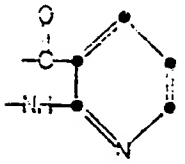
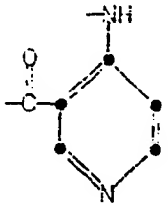
in which R^8 is hydroxymethyl, hydroxyethyl, mercaptomethyl, mercaptoethyl, methylthioethyl, 2-thienyl, 3-indole-methyl, phenyl, benzyl, or substituted phenyl or substituted benzyl in which the benzene ring thereof is substituted with chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3-alkoxy, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

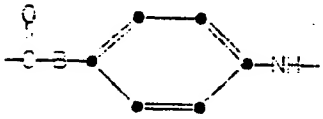
(c) 

in which X is hydrogen, chloro, bromo, iodo, amino, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy, mercapto, C_1-C_3 alkylthio, carbamyl, or C_1-C_3 alkylcarbamyl;

(d)  or 

in which X^1 is chloro, bromo, iodo, amino, hydroxy, C_1-C_3 alkyl or C_1-C_3 alkoxy;

(e)  or  ; or

(f) 

in which B is a divalent radical of the formula: $-(CH_2)_n-$ and n is an integer from 1 to 3; -CH=CH-; $-CH=CH-CH_2-$; or

$-CH_2-NH-\overset{\overset{O}{\|}}{C}-$;

R^7 is C_1-C_{17} alkyl or C_2-C_{17} alkenyl; and R^2 is hydrogen, an amino-protecting group, an aminoacyl group of the formula $-\overset{O}{\overset{\|}{C}}-Q-NH_2$ as herein defined, or an N-alkanoylaminoacyl group of the formula $-W-\overset{O}{\overset{\|}{C}}-R^7$ as herein defined; or a pharmaceutically-acceptable salt thereof.

3. A compound according to claim 2 in which R^1 and R^2 are hydrogen and R is selected from 5-[N-(n-dodecanoyl)amino]-n-pentanoyl, m-[N-(n-dodecanoyl)-amino]benzoyl, p-[N-(n-dodecanoyl)amino]cinnamoyl, 2-[N-(n-dodecanoyl)amino]nicotinoyl, or N-(n-decanoyl)-phenylalanyl; or a pharmaceutically-acceptable salt thereof.

4. An A-21978C cyclic peptide of formula (1) as claimed in claim 1 in which R is selected from the group consisting of hydrogen, an amino-protecting group, 8-methyldecanoyl, 10-methylundecanoyl, 10-methyldodecanoyl, the specific C_{10}-alkanoyl group of A-21978C factor C_0 and the specific C_{12}-alkanoyl groups of A-21978C factors C_4 and C_3; R^1 and R^2 are, independently, hydrogen or an amino-protecting group; provided that, when R is other than hydrogen or an amino-protecting group, at least one of R^1 and R^2 must be an amino-protecting group; or a pharmaceutically-acceptable salt thereof.

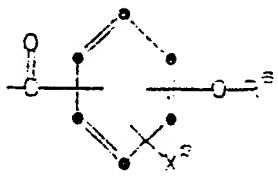
5. A compound according to claim 4 in which R is hydrogen or a pharmaceutically-acceptable salt thereof.

6. A compound according to claim 5 in which R^2 is hydrogen or a pharmaceutically-acceptable salt thereof.

7. A compound according to claim 5 or 6 in which R^1 is hydrogen or a pharmaceutically-acceptable salt thereof.

8. A compound according to claim 5 in which R^2 is an amino-protecting group or a pharmaceutically-acceptable salt thereof.

9. An A-21978C cyclic peptide derivative of formula (1) as claimed in claim 1 in which R is a substituted benzoyl group of the formula



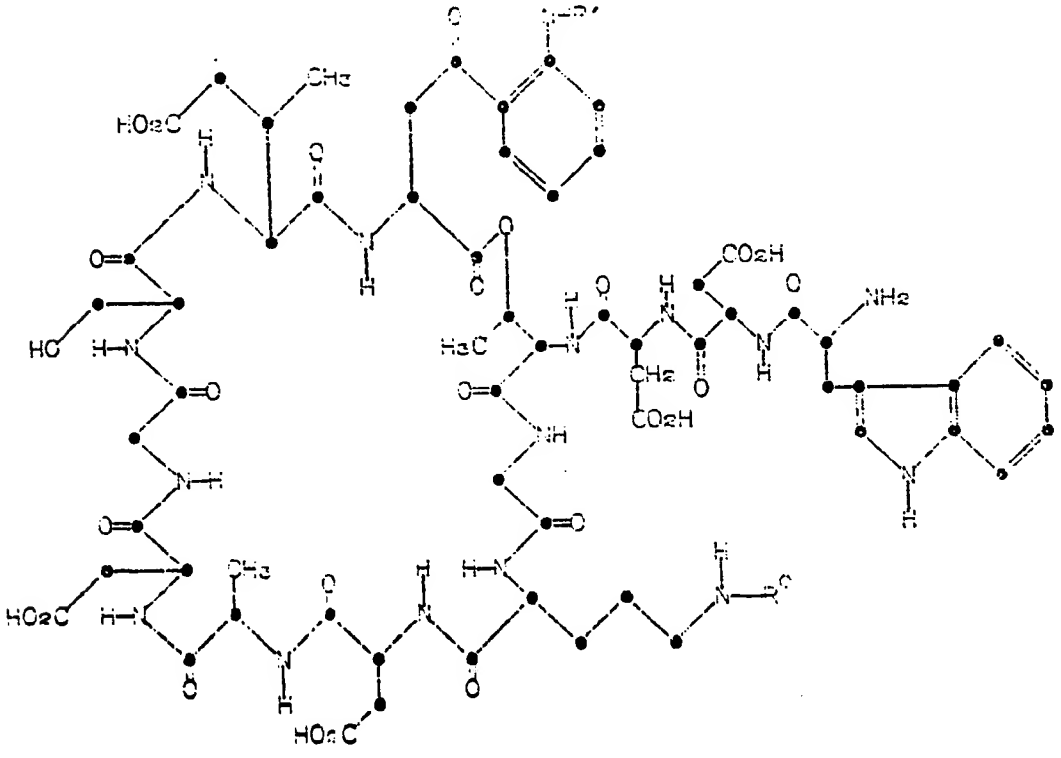
in which R^6 is C_8-C_{15} alkyl; X is hydrogen, chloro, bromo, iodo, nitro, C_1-C_3 alkyl, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkylthio; and R^2 is hydrogen or an amino-protecting group; or a pharmaceutically-acceptable salt thereof.

10. An A-21978C cyclic peptide derivative of formula (1) as claimed in claim 1 in which R, R^1 and R^2 are, independently, hydrogen, C_4-C_{14}-alkyl, optionally substituted C_2-C_{19}-alkanoyl, C_5-C_{19}-alkenoyl or an amino-protecting group, R^3, R^4 and R^5 are all hydrogen, or (i) R^3 and R^1; and/or (ii) R^4 and R; and/or (iii) R^5 and R^2, taken together may represent a C_4-C_{14} alkylidene

group, provided that the R, R^1 and R^2 groups must together contain at least four carbon atoms; or a pharmaceutically-acceptable salt thereof.

11. A compound according to claim 10 in which R^1 and R^2 are hydrogen and R is selected from 9-tetradecenoyl, 10-undecenoyl, or dodecyl or a pharmaceutically-acceptable salt thereof.

12. A process for preparing an A-21978C cyclic peptide derivative of formula (1) as claimed in any one of claims 2, 3 or 9 which process comprises acylating an A-21978C cyclic peptide factor, the A-21978C nucleus of formula (3),



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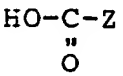
or an appropriately protected derivative thereof in which R' or R^O are, independently, hydrogen or an amino-protecting group or a pharmaceutically-acceptable salt thereof, with an acylating agent of formula (5)



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in which Z is $-Q-NH_2$, $-A-NH-\overset{\overset{O}{\|}}{C}-R^7$, $-\underset{R^8}{CH}-NH-\overset{\overset{O}{\|}}{C}-R^7$,

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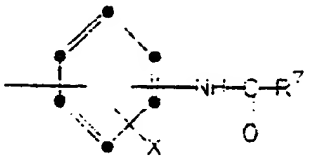

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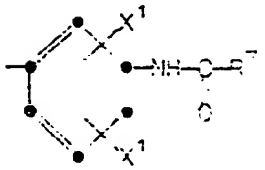

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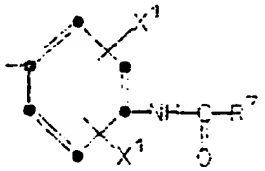

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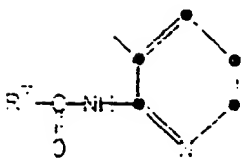

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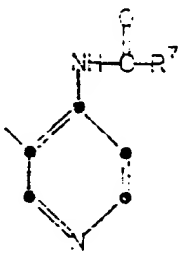

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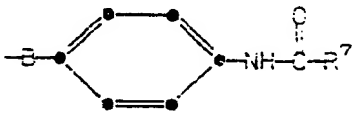

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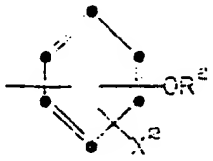
or



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or an activated derivative thereof, in which Q, A, R^6, R^7, R^8, X, X^1, X^2, and B are as previously defined, and optionally, if desired, removing any protecting group which may be present in the product of the reaction.

13. A process for preparing an A-21978C cyclic peptide as claimed in any of claims 4 to 8 which process comprises

(a) the protection of one or more of the amino groups NHR, NHR^1 and NHR^2 so as to prepare a compound of formula (1) in which one or more of R, R^1 and R^2 represent an amino-protection group, provided that one or more of R, R^1, and R^2 were initially hydrogen, and/or

(b) the deacylation of a compound of formula (1) in which R is other than hydrogen or an amino group, so as to prepare a compound of formula (1) in which R is hydrogen, and, optionally, if so desired, removing any R^1 or R^2 amino protecting groups which may be present in the product of the reaction.

14. A process according to claim 13 in which the method of deacylation comprises exposing the compound of formula 1 in an aqueous medium to an enzyme which deacylates and which is produced by a microorganism of the family Actinoplanaceae until substantial deacylation is accomplished.

15. A process according to claim 14 in which the microorganism of the family Actinoplanaceae is a member of the genus Actinoplanes.

16. A process according to claim 15 in which the microorganism is selected from A. utahensis NRRL 12052, Streptosporangium roseum var. hollandensis NRRL 12064, Actinoplanes missouriensis NRRL 12053, Actinoplanes sp. NRRL 12065, or Actinoplanes sp. NRRL 8122; or a mutant or variant thereof.

17. A process as claimed in any one of claims 14 to 16 in which the enzyme is present in a culture of the producing Actinoplanaceae microorganism.

18. A process for preparing an A-21978C cyclic peptide derivative of formula 1 as claimed in claims 10 or 11 which comprises reacting an A-21978C cyclic peptide nucleus of formula 3, as defined in claim 12,

(a) with an acylating agent of formula (7):

$$R^{10}\text{-COOH} \qquad (7)$$

or an activated derivative thereof, in which R^{10} is optionally substituted C_1-C_{18} alkyl or C_4-C_{18} alkenyl; or

(b) with a carbonyl derivative of formula (8):

$$R^{11}\text{-}\underset{\underset{O}{\|}}{C}\text{-}R^{12} \qquad (8)$$

in which the $R^{11}R^{12}C=$ group represents a C_4-C_{14} alkylidene grouping; and

(c) optionally reducing a C_4-C_{14} alkylidene product of the above process (b) to form a compound of formula I in which R, R^1 or R^2 represent a C_4-C_{14} alkyl group;

(d) and, if desired, removing any protecting group which may be present in the product of any of reactions (a), (b), or (c) above.

19. A pharmaceutical formulation which comprises as an active ingredient an A-21978C derivative of formula (1), or a pharmaceutically-acceptable salt thereof, as claimed in any one of claims 1 to 11, associated with one or more physiologically-acceptable carriers or vehicles therefor.

20. An A-21978C derivative of formula (1), or a pharmaceutically-acceptable salt thereof, as claimed in any one of claims 1 to 11, for use as an antibiotic.

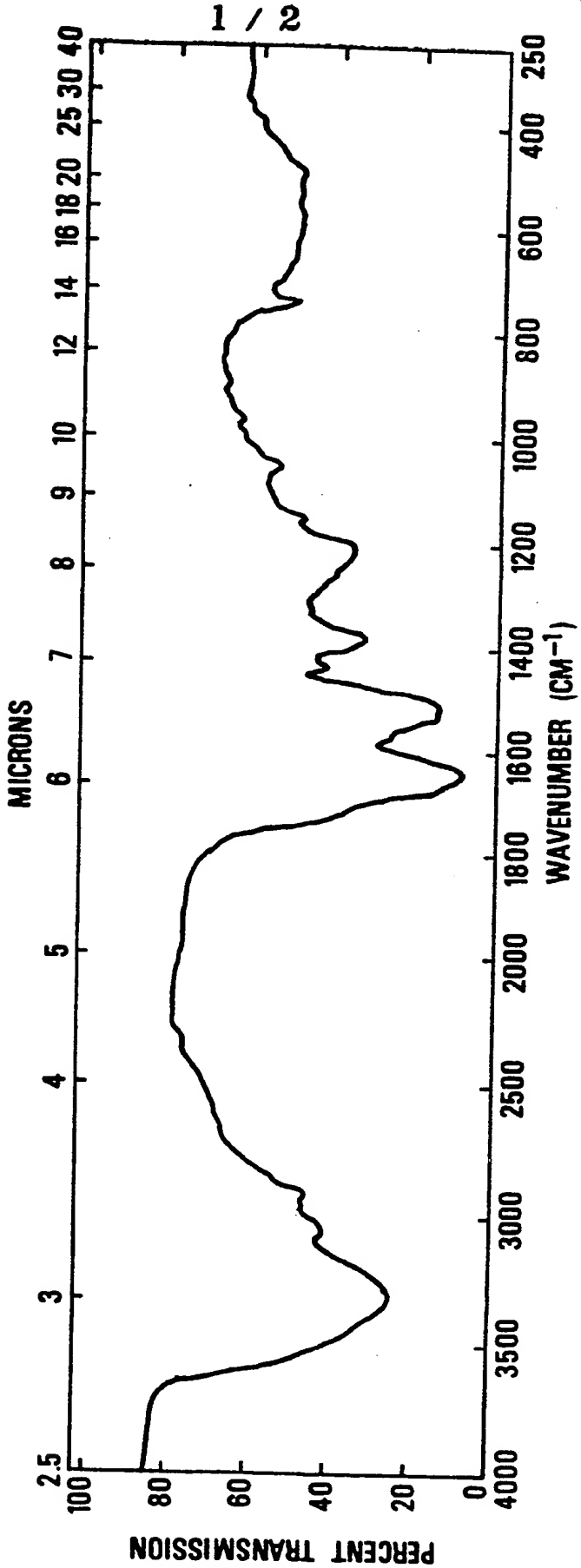

FIG. 1

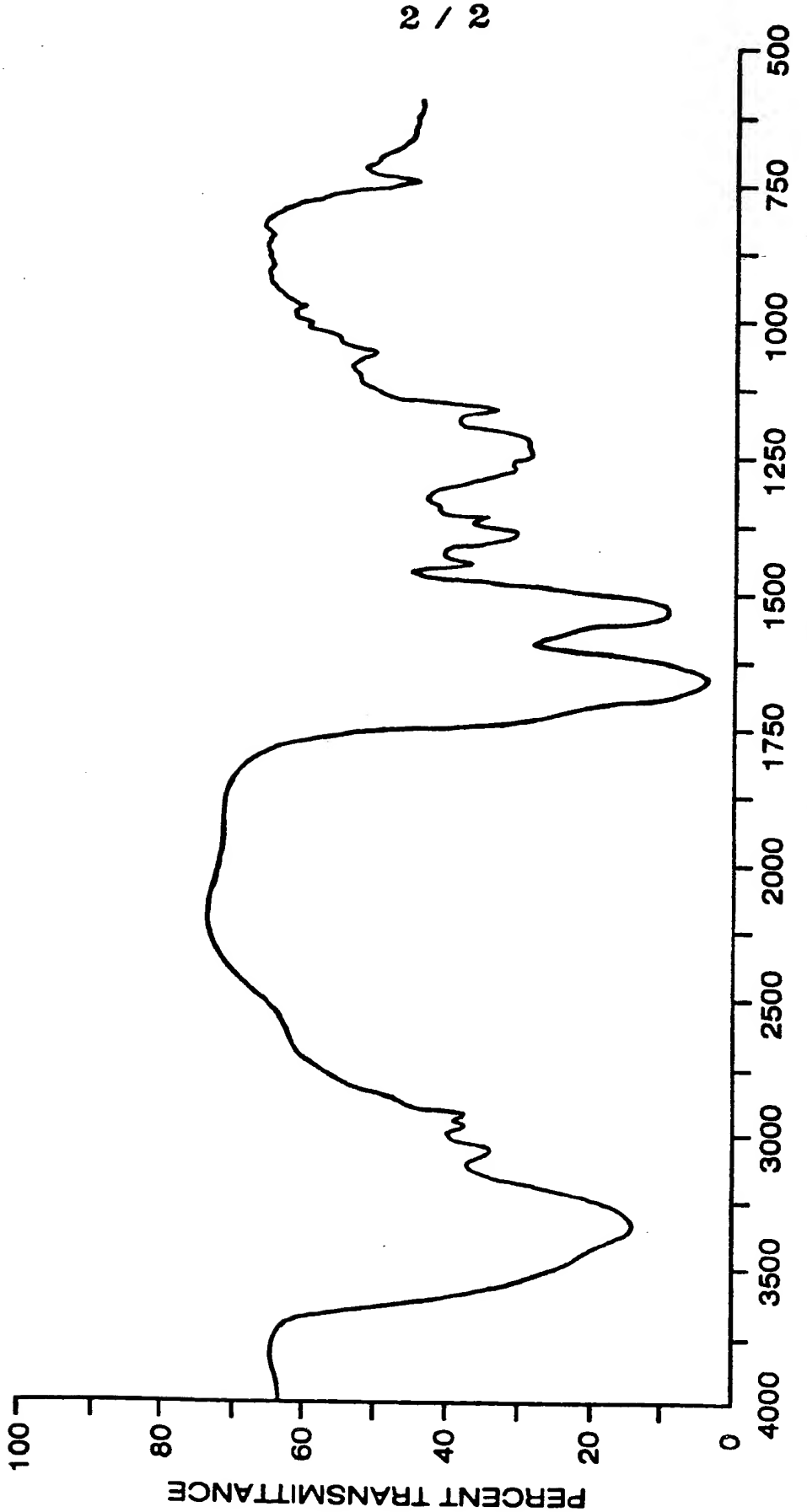
PERCENT TRANSMITTANCE
100
80
60
40
20
0
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
WAVENUMBERS

FIG. 2

European Patent Office

EUROPEAN SEARCH REPORT

0095295
Application number

EP 83 30 2744

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
D,Y	EP-A-0 010 421 (E. LILLY) * Whole document * ---	1,2,3-5,19	C 07 C 103/52 C 12 P 21/04 A 61 K 37/02 // (C 12 P 21/04 C 12 R 1/045 C 12 R 1/62)
Y	EP-A-0 031 221 (E. LILLY) * Whole document * ---	1,12, 14-17, 19	
P,Y	EP-A-0 055 070 (E. LILLY) * Whole document * -----	1,12, 14-17, 19	

TECHNICAL FIELDS SEARCHED (Int. Cl. ³)

A 61 K 37/00
C 07 C 103/00
C 12 P 21/00
C 12 R 1/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	11-08-1983	RAJIC M.

CATEGORY OF CITED DOCUMENTS

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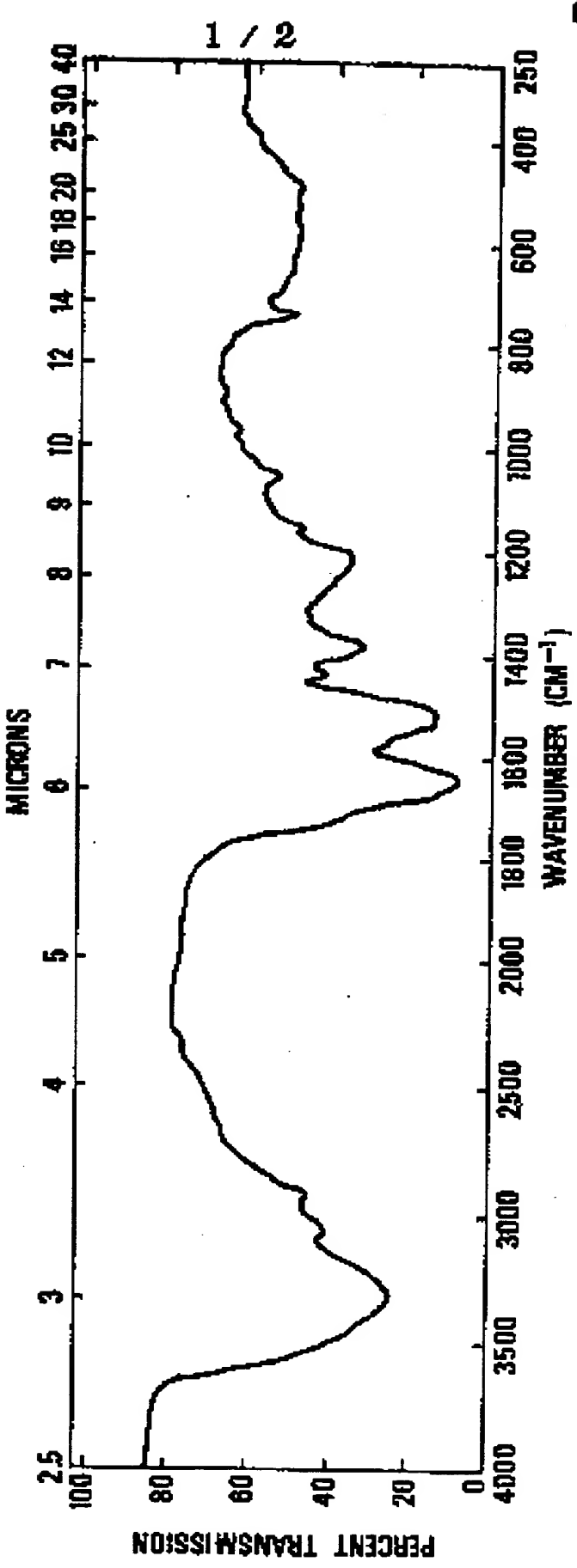

FIG. 1

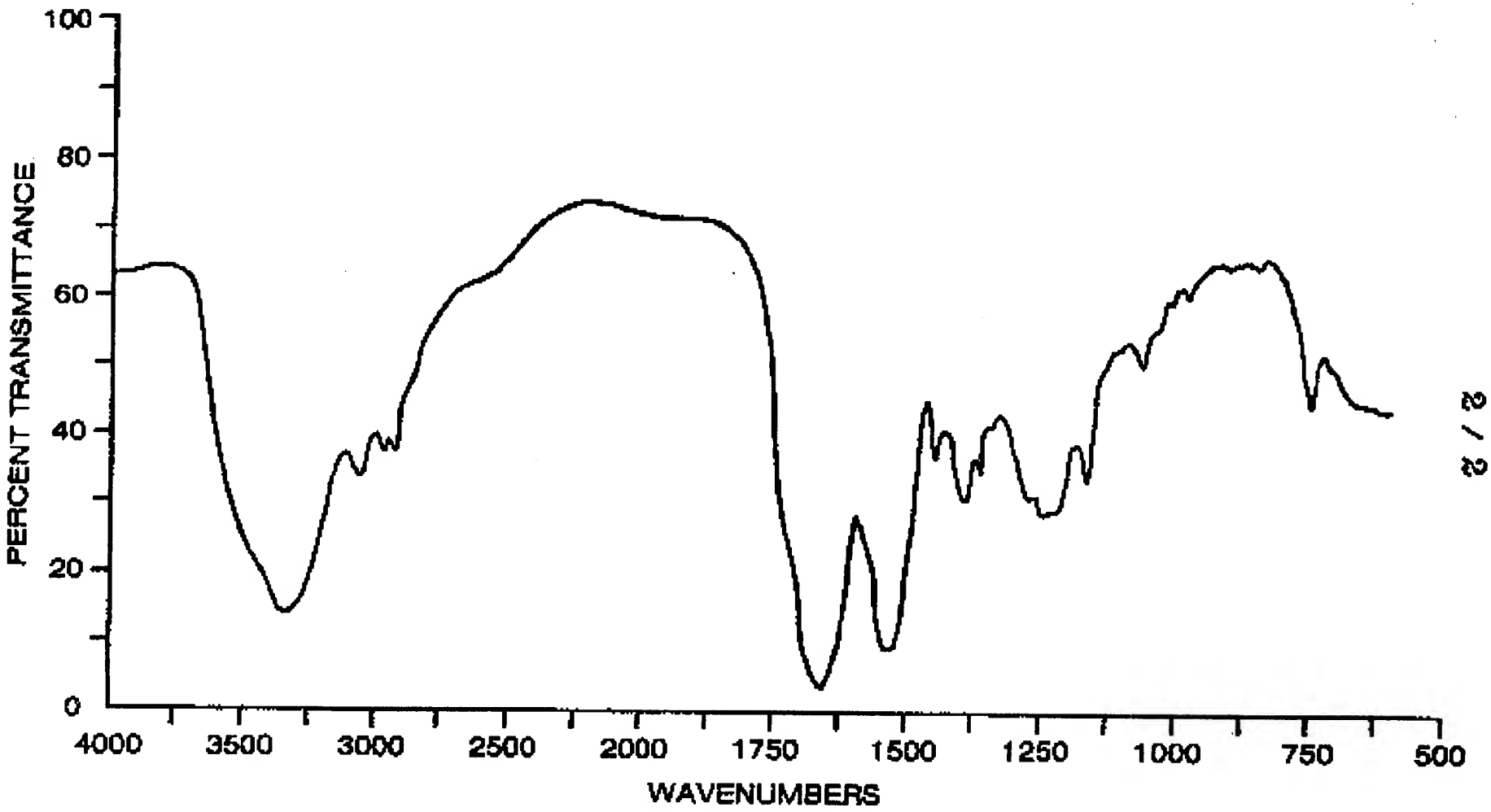

FIG. 2